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(57) Abstract

UNC-53 protein of C. elegans or its functional equivalent is identified as a signal transducer/integrator involved in controlling the rate and directionality of cell migration and/or cell shape. Nucleic acid sequences encoding UNC-53 protein or its functional equivalent, such as genomic or cDNA are used to transfect C. elegans or mammalian cell lines useful for identifying inhibitors or enhancers of the UNC-53 protein. Any of the inhibitors or enhancers identified or the UNC-53 protein itself or sequences encoding UNC-53 protein can be used in the preparation of medicament for treatment of neurological conditions such as Alzheimer's or Huntingdon's disease, peripheral neuropathies for inhibition of metastasis.

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PROCESSES FOR THE IDENTIFICATION OF COMPOUNDS WHICH CONTROL CELL BEHAVIOUR, THE COMPOUNDS IDENTIFIED AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND THEIR USE IN THE CONTROL OF CELL BEHAVIOUR

The present invention relates to processes for the identification of compounds which inhibit or enhance the rate and direction of cell migration or the control of cell shape, the compounds identified and pharmaceutical formulations containing such compounds together with their use in the regulation of cell behaviour. The invention also relates to an UNC-53 protein encoded by nucleic acid in the cells of the nematode worm C. elegans and cDNA sequences encoding an UNC-53 protein or functional equivalents thereof.

The control of cell motility, cell shape and the outgrowth of axones or other cell outgrowths is an essential feature in the morphogenesis and function of both unicellular and multicellular organisms. The control of this process is disturbed in a variety of disease states in which for example the Receptor Tyrosine Kinase (RTK) signal transduction pathways or the like or their downstream intra-cellular pathways (which are shared with other extra-cellular receptors, including cell adhesion molecules like N-CAMS and integrins) are overstimulated.

Some cell surface proteins and extracellular molecules controlling the directionality and potential of cell migration have been identified. However the processes in which these proteins or molecules are involved to effect cell migration, shape or rate of cell differentiation are not understood.

It is generally considered that a long-range migration of a cell process (which may also be known as a growth cone spike) is a stepwise event, whereby prior to and after each extension there is the

formation of a structure at the leading edge of the cell which senses signals in the environment instructing the cell to either stabilize a cell process extending in a preferred direction, or to cause a cell process lamellipodium to extend a process in a given direction. Localized stabilization of the actin cytoskeleton, is a general cell biological process underlying this choice of directional extension.

A gene from the free-living nematode Caenorhabditis elegans, designated "unc-53" has been previously identified and cloned (Abstract, International C. elegans meeting; June 1-5 1991, Madison, Wisconsin, 58, Bogaert and Goh). However, to date no known biological function has been attributed to the unc-53 gene or its corresponding UNC-53 protein.

The present inventors have surprisingly identified, through biochemical, genetic, phenotypic and transgenic evidence which is presented herewith, UNC-53 as a signal transducer or signal integrator controlling the rate and directionality of cell migration, and/or cell shape. Key experiments leading to this conclusion were the molecular identification of its domain structure, its biochemical interaction with GRB-2, actin cytoskeleton sequence information and the presence of a potential signal integrating domain in the UNC-53 protein.

An additional key observation is that increased UNC-53 protein activity is proportional to increased cell process extension in the correct direction of cell migration. Reduction of UNC-53 function has previously been shown to lead to a reduction of cell process extension, identifying it as a general component required for cell migration. However, it had not been identified as a component whose level of

activity has a determining role in the specification of the quantum and directionality of migration.

The work of the present inventors suggests that UNC-53 plays a central role in quantitatively transducing extracellular signals to the machinery controlling directional cell migration.

The importance of UNC-53 in a variety of cell types in C. elegans has been demonstrated. The gene encodes a signal transduction molecule that transduces a signal from a Receptor Tyrosine Kinase such as for example via the adaptor protein SEM-5/GRB-2, to the machinery controlling directional growth cone extension or stabilization. The UNC-53 protein does this in a highly dosage-dependent fashion whereby reduction of protein activity such as reduction in expression of protein or in the reduction in its activity leads to proportional reduction of cell process extension (cell migration). This is believed to be either by regulated cross-linking of the actin cytoskeleton or by transferring the received signal downstream within the transduction pathway. Higher than wild type UNC-53 expression leads to higher than wild type growth cone extension in the anterior-posterior axis. Both the observed SEM-5/GRB-2 binding to UNC-53 and the predicted ATP/GTP-ase activity of UNC-53 demonstrate a signal transduction role for UNC-53 involved in cell process or growth cone guidance.

UNC-53 is a protein working at the intracellular level. It is so far believed to be the only intracellular protein identified which is involved in the control of directionality and rate of cell migration in response to a specific signal and which integrates different directional signals in defining direction of migration.

Based on the present inventors accumulated

knowledge of the unc-53 gene function in C. elegans it is understood that inhibitors or enhancers of the unc-53 gene or the UNC-53 protein will affect the cell motility including (metastasis) via an RTK pathway or the like, or may lead to changes in the shape of the cells (which has been demonstrated in C. elegans body muscle). Applications for such inhibitors and/or enhancers are envisaged in a wide variety of pathologies in which the RTK pathways play a central role, including oncogenesis, psoriasis, cell migration (metastasis), neuronal regeneration/degeneration and immunological disorders among others.

The identification of the biochemical function of the unc-53 gene (and UNC-53 pathway) in the RTK signal transduction pathway is novel and unexpected. No biological function has previously been linked to the unc-53 gene or UNC-53 protein, nor has any homology with any other nucleic acid sequence or gene been recognised.

An analysis of the predicted protein sequence of UNC-53 from the gene sequence thereof has revealed the following:

(a) an N-terminal domain with homology to cortical actin binding proteins of the α-actinin and β-spectrin families (designated ABPII in Figure 11). Alignment of UNC-53 with the α-actinin and β-spectrin family of proteins is shown in Fig. 15.).

(b) two putative actin binding sites of the LKK class (ABS1 and ABS2).

(c) two polyproline rich sequences similar to the SH3 binding domains of the SOS family of signal transduction molecules (SH3 binding site) (Fig. 16).

(d) a putative ATP/GTP nucleotide binding site having some of the additional features of the GTP

binding domain of RAS-like proteins (Dynamin, NBD).

(e) besides the N-terminal region of the protein, which is similar to actin binding proteins, the predicted protein sequence of UNC-53 identified two putative actin binding sites. The first borders on the 3′ end of the region of α-actinin/β-spectrin homology and the second lies in the 3′ end of the cDNA sequence.

This suggests that UNC-53 could potentially bind two actin molecules and via actin cross linking, could stabilize a particular cell process to promote directional extension.

In addition, genetic evidence shows that alleles of unc-53 enhance the sex myoblast migration defect of sem-5 mutants. Sem-5 represents the C. elegans homologue of GRB2, the function of these proteins being assigned/attributed to their SH2 and SH3 domains (Clark et al., (1992) Nature 356, 340-344; Stern et al., (1993), Molec. Biol. Cell, 4, 1175-1188). The current model regarding sem-5 function in the migration of sex myoblasts is that sem-5 transduces a signal received at the cell surface by egl-15, a receptor kinase of the fibroblast growth factor family. Together, the genetic and molecular data suggest a role for UNC-53 in both signal transduction and actin binding. We have been able to demonstrate how UNC-53 might act to direct both growth cone rate and directionality. By binding directly to the actin cytoskeleton, UNC-53 may stabilize and cross-link actin molecules (assuming a two actin binding site model) to promote directional growth cone extension. Alternatively, by binding actin, UNC-53 may convey a signal to the cytoskeleton and then via an ATP/GTPase activity transduce the signal to downstream targets. To test these models, biochemical experiments were

conducted to determine if any of the sequence similarities observed represented functional domains (see examples 2 to 5). Transgenic analysis as described in examples 6 to 8 support this proposed model.

As described above, the unc-53 gene from C. elegans has been previously identified. However, cDNA sequences substantially corresponding to unc-53 genomic exon sequences of C. elegans or fragments or derivatives thereof have never been previously disclosed. The present inventors have advantageously identified two unc-53 cDNA clones which have been designated as the 7A and 8A clones. The two clones differ in the number of Adenosine(A) residues (7 or 8) in a poly A stretch of the 3′ coding region. Therefore, the two clones have different reading frames in the carboxyterminal coding region.

Therefore according to one aspect of the present invention there is provided a cDNA encoding an UNC-53 protein of C. elegans or a functional equivalent derivative or bioprecursor of said protein which cDNA comprises at least from nucleotide position 431 to nucleotide position 4647 or alternatively to the 3′ poly-A region of the sequence shown in Figure 1. More preferably the cDNA comprises at least from nucleotide position 64 to nucleotide position 4647 or to the 3′ poly-A region of the sequence as shown in Figure 1. This cDNA is comprised in the 8A clone having 8A residues in a poly A stretch of the 3′ coding region as shown in Figure 1.

In an alternative embodiment of this aspect of the invention the cDNA comprises at least from nucleotide position 431 to nucleotide position 4812 or alternatively to the 3′ poly-A region of the sequence shown in Figure 2 and more preferably at least from position 64 to nucleotide position 4812 or the 3′

poly-A region of the sequence shown in Figure 2. This cDNA according to the invention comprises the 7A clone, having only 7 Adenine residues in the poly A stretch of the 3′ coding region as shown in the nucleotide sequence of Figure 2 page 8. Each of the cDNA clones according to the invention, may be included in an expression vector which vector may itself be used to transform or transfect a host cell which may be bacterial, animal or plant in origin. Thus, advantageously, once the cDNA corresponding to the unc-53 genome is synthesised using for example reverse transcriptase or the like, a range of cells, tissues or organisms may be transfected following incorporation of the selected cDNA clone into an appropriate expression vector.

The present invention therefore, also further comprises a transgenic cell, tissue or organism comprising a transgene capable of expressing UNC-53 protein of C. elegans or a functional equivalent, fragment, derivative or bioprecursor thereof. The term "transgene capable of expressing UNC-53 protein" as used herein means a suitable nucleic acid sequence which leads to the expression of an UNC-53 protein having the same function and/or activity. The transgene may include for example genomic nucleic acid isolated from C. elegans or synthetic nucleic acid or alternatively any of the cDNA clones as described above.

The term "transgenic organism, tissue or cell" as used herein means any suitable organism and/or part of an organism, tissue or cell that contains exogenous nucleic acid either stably integrated in the genome or in an extra chromosomal state.

Preferably, the transgenic cell comprises either a C. elegans cell, an N4 neuroblastoma cell or an MCF-7 breast carcinoma cell. The transgenic organism may

be C. elegans itself, or alternatively may be an insect, a non-human animal or a plant. Preferably the unc-53 transgene comprises the unc-53 gene or a functional fragment thereof. The term "functional fragment" as used herein should be taken to mean a fragment of an UNC-53 gene which encodes an UNC-53 protein or a functional equivalent or bioprecursor of the protein. For example the gene may comprise deletions or mutations but may still encode a functional UNC-53 protein.

Reference to "tissue or tissue culture" for the purpose of the present invention should be taken to mean such a mutant cell which has been grown in such a culture. Further provided by the present invention is a mutant C. elegans organism which comprises an induced mutation, such as a point mutation in the wild-type unc-53 gene and which mutation affects the regulation of cell motility or shape or the direction of cell migration. Such mutations may be introduced using changes in the cDNA corresponding to qualitative, quantitative direct and indirect changes in the genomic make up.

The term "mutant organism" used herein means any suitable organism that contains genetic information which has been induced to mutate and is thus altered from the wild-type. Therefore naturally occurring mutations in the wild-type organism are not within the scope of this term.

The present invention further comprises an UNC-53 protein or a functional equivalent or fragment thereof, which protein may be encoded by a cDNA according to the invention, and which protein has the amino acid sequence shown in Figure 4 from amino acid position 135 to amino acid position 1528; this corresponds to the 8A clone. More preferably the UNC-53 protein, when encoded by a cDNA according to the

invention, comprises the amino acid sequence shown in Figure 4. In another aspect of the invention the protein comprises an UNC-53 protein or a functional equivalent, fragment or bioprecursor of the protein which comprises the sequence of from amino acid position 135 to amino acid position 1583 of the amino acid sequence shown in Figure 6. Preferably, the UNC-53 protein when encoded by a cDNA in accordance with the invention has the amino acid sequence shown in Figure 6.

The UNC-53 protein of C. elegans or a functional equivalent, fragment or bioprecursor of the UNC-53 protein, may advantageously be used as a medicament to promote neuronal regeneration, revascularisation or wound healing or the treatment of chronic neuro-degenerative disorders or acute traumatic injuries. Similarly, the UNC-53 protein produced by the transgenic cells, tissue or organisms according to the invention may also be used in the preparation of a medicament for treatment of the conditions as described above.

Furthermore, in an alternative embodiment of the invention the nucleic acid sequence itself encoding an UNC-53 protein of C. elegans or a functional equivalent, fragment or bioprecursor of the protein may also be used as a medicament or, alternatively in the preparation of a medicament, to promote neuronal regeneration, vascularisation or wound healing or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries. Typically neurological conditions which may be treated by either an UNC-53 protein or a functional equivalent thereof, or a nucleic acid according to the invention, comprise peripheral nerve regeneration after trauma; recovery of function of the spinal cord after spinal cord trauma or peripheral neuropathies. Similarly neuro-

degeneration diseases which may be treated include Alzheimers disease or Huntingdons disease. Acute traumatic injuries such as stroke, head trauma or haemorrhages may also advantageously be treated.

The nucleic acid sequence according to the invention may comprise a cDNA sequence according to the invention as described above or alternatively may be genomic DNA derived from C. elegans.

The UNC-53 protein of C. elegans, or a functional equivalent, fragment or bioprecursor of said protein may be incorporated into a pharmaceutically acceptable composition together with a suitable carrier, diluent or an excipient therefor. The pharmaceutical composition may advantageously comprise, additionally or alternatively to the UNC-53 protein according to the invention, the nucleic acid sequence according to the invention as defined above.

The present invention also provides for a method of determining whether a compound is an inhibitor or an enhancer of the regulation of cell shape or motility or the direction of cell migration in a transgenic cell, tissue or organism according to the invention as described herein. The method preferably comprises contacting the compound with a transgenic cell, tissue or organism according to the invention as described above, and screening for a phenotypic change in the cell, tissue or organism. Preferably the compound comprises an inhibitor or enhancer of a protein of the signal transduction pathway of the cell, tissue or organism of which UNC-53 is a component or is an inhibitor or enhancer of a parallel or redundant signal transduction pathway. Such enhancers or inhibitors are defined by particular phenotypic changes in the transgenic cell, tissue or organism, for example changes in cell shape or mobility or the direction of cell migration.

Preferably the compound is an inhibitor or an enhancer of the activity of UNC-53 protein of C. elegans or a functional equivalent, derivative or bioprecursor thereof, which protein is expressed in the transgenic cell, tissue or organism as defined herein.

Preferably the phenotypic change to be screened comprises a change in cell shape or a change in cell motility. Where a transgenic cell is used in accordance with one embodiment of the method of the invention, an N4 neuroblastoma cell may be used and in such an embodiment the phenotypic change to be screened may be the length of neurite growth or changes in filipodia outgrowth or alternatively changes in ruffling behaviour or cell adhesion. In an alternative embodiment of the method of the invention, the transgenic cell may comprise an MCF-7 breast carcinoma cell. Typically in such an embodiment the phenotypic change to be screened comprises the extent of phagokinesis. The method according to the invention, may also utilise a mutant cell or mutant organism according to the invention as described above, where the mutant cell is capable of growing in tissue culture and either of which cell or organism has a mutation in the wild-type unc-53 gene.

In accordance with the present invention, a "phenotypic change", may be any phenotype resulting from changes at any suitable point in the life cycle of the cell, tissue or organism defined above, which change can be attributed to the expression of the transgene such as for example, growth, viability, morphology, behaviour, movement, cell migration or cell process or growth cone extension of cells and includes changes in body shape, locomotion, chemotaxis, mating behaviour or the like. The phenotypic change may preferably be monitored directly by visual inspection or alternatively by for example

measuring indicators of viability including endogenous or transgenically introduced histochemical markers or other reporter genes, such as for example ß-galactosidase.

A compound which is identifiable by the method according to the invention as described above, as an enhancer of the regulation of cell shape or motility or the direction of cell migration in C. elegans may be used as a medicament, or alternatively in the preparation of a medicament, for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries. Examples of promoting neuronal regeneration include for example peripheral nerve regeneration after trauma and spinal cord trauma.

Where a compound is identified in accordance with the method described above as being an inhibitor of the regulation of cell shape, the compound may be used as a medicament, or in the preparation of a medicament, for substantially alleviating spread of disease inducing cells, such as in spread of cancers, or the like in metastasis. Advantageously, any of the compounds which may have been identified as an inhibitor or an enhancer in accordance with the method as described above, may also be included in a pharmaceutically acceptable formulation comprising the respective compound and an acceptable carrier, diluent or excipient therefor.

The particular mechanism of action of a compound identified as either an inhibitor or an enhancer of the cell motility or direction of cell migration is not limiting preferably the compound acts as an inhibitor or enhancer of a signal transduction pathway downstream. The compound may also act on parallel pathway or on the UNC-53 protein of C. elegans. For

example, the method of action of the compound may include direct interaction with UNC-53 protein, interaction with processes for regulating phosphorylation of UNC-53 or for processes regulating activity of an unc-53 gene or for processes for post-transcriptional or post-translational modification or the like.

Preferably the compound is identified by the method according to the invention as an inhibitor or an enhancer, by utilising differences of phenotype of the cell, tissue or organism, which are visible to the eye. Alternatively indicators of viability including endogenous or transgenically introduced histochemical markers or a reporter gene may be used.

According to a further aspect of the invention there is also provided a transgenic cell or tissue culture which has been constructed to comprise a promoter sequence of an unc-53 gene of C. elegans or a functional fragment thereof, fused to a nucleic acid sequence encoding a reporter molecule. Preferably, the reporter sequence encoding the reporter molecule encodes for a detectable protein, for example one which may be monitored by eye inspection such as antibiotic resistance, ß-galactosidase or a molecule detectable by spectrophotometric, spectrofluorometric, luminescent or radioactive assays. Preferably the reporter molecule is green fluorescent protein (GFP), which advantageously allows inhibition or enhancement of the UNC-53 protein according to the invention to be monitored visually.

The present invention also provides a method of determining whether a compound is an inhibitor or an enhancer of transcription of a an unc-53 gene in C. elegans, or a functional fragment thereof, which method comprises the steps of:

(a) contacting said compound with a transgenic

cell according to the further aspect of the invention as described above,

(b) monitoring the reporter molecule and comparing results obtained from this monitoring step with a control comprising a transgenic cell having the promoter sequence of an unc-53 gene, or a functional fragment thereof and the reporter molecule, in the absence of the compound.

In one embodiment of the method according to the invention the reporter molecule may comprise messenger RNA. Alternatively the reporter molecule may be green fluorescent protein (GFP).

A compound identified as an inhibitor or enhancer of transcription of the unc-53 gene or a fragment thereof may also be used as a medicament, or in the preparation of a medicament, for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries. Furthermore, such compounds may be included in a pharmaceutical formulation including a carrier, diluent or excipient therefor.

The present invention also provides a kit for determining whether a compound is an enhancer or an inhibitor of the regulation of cell motility or shape or the direction of cell migration, which kit comprises at least a plurality of transgenic or mutant cells according to the invention as described above and a plurality of wild-type cells of the same cell type or cell line or tissue culture.

Also provided by the present invention is a kit for determining whether a compound is an inhibitor or an enhancer of transcription of an unc-53 gene of <u>C. elegans</u> or a functional fragment thereof, which comprises at least a plurality of transgenic cells as described above and means for monitoring the reporter

molecule.

For the purposes of the present invention, the term "unc-53 gene or a functional fragment thereof" includes the nucleic acid sequence shown in Figure 1 or a fragment thereof, including the differentially spliced isoforms and transcriptional start of the unc-53 gene sequence and which sequence encodes an UNC-53 protein or a functional equivalent, derivative, fragment or bioprecursor of the protein.

The present invention also provides an oligonucleotide probe which comprises the carboxy-terminal 1.5 kb of the coding nucleic acid sequence shown in Figure 1 or a fragment thereof comprising not less than 15 base pairs. In addition, the present invention provides a further oligonucleotide probe comprising a nucleic acid sequence encoding the amino acid sequence as numbered 1 to 10 and 14 to 133, 487 to 495, 537 to 545, 1032 to 1037, 1097 to 1116 or 1300 to 1307, as shown in Figure 3 or a fragment thereof comprising between 18 and 24 base pairs. The oligonucleotide probes described above may also be advantageously be labelled for detection.

The present invention also provides methods of identifying C. elegans genes or fragments thereof, which encode proteins which are active in the signal transduction pathway of which UNC-53 is a component and which are homologues of UNC-53. A preferred method comprises hybridizing to a C. elegans cDNA library an oligonucleotide probe according to the invention as described above, under appropriate conditions or stringency in order to identify genes having statistically significant homology with the cDNA clones of any one of the cDNA sequences according to the invention described above.

Furthermore, there is also provided by the present invention a method of identifying a protein

which is active in the signal transduction pathway of a cell. According to this aspect of the invention, the method comprises;

(a) contacting an extract of said cell with an antibody to the UNC-53 protein or a functional equivalent, fragment or bioprecursor thereof,

(b) identifying the antibody/UNC-53 complex, and

(c) analysing the complex to identify any protein bound to the UNC-53 protein other than the antibody.

The UNC-53 protein, therefore may bind regions of other proteins involved in the signal transduction pathway. It is also possible to sequentially identify a whole range of proteins involved in the signal transduction pathway. This aspect of the invention, further comprises a method of identifying a further protein or proteins which are active in the signal transduction pathway of a cell which method comprises:

(a) forming an antibody to the identified protein bound to the UNC-53 protein in the method as described above,

(b) contacting a cell extract of C. elegans with the antibody,

(c) identifying the antibody/protein complex,

(d) analysing the complex to identify any further protein bound to the first protein other than the antibody, and

(e) optionally repeating steps (a) to (d) to identify further proteins in the pathway.

According to this aspect of the present invention, the antibody, which is preferably a monoclonal antibody, such as for example monoclonal antibody designated as 16-48-2, starts the process by binding to the UNC-53 protein or a functional equivalent thereof in the signal transduction pathway. Any other proteins found complexed to the bound

antibody or UNC-53 protein can then be used to identify further interacting proteins involved in the pathway.

It may also be possible to identify proteins involved in the signal transduction of a cell by using UNC-53 protein of C. elegans. According to this aspect of the invention the method comprises:

(a) contacting an extract of the cell with the UNC-53 protein of C. elegans or a functional equivalent, fragment or bioprecursor of said UNC-53 protein

(b) identifying the UNC-53 protein/protein complex and

(c) analysing the complex to identify any protein bound to the UNC-53 protein other than another UNC-53 protein

This method can also advantageously be used to identify further proteins in a signal transduction pathway of a cell by contacting an extract of the cell used as described above, with any protein identified from step (c) above not being an UNC-53 protein and repeating steps (b) and (c).

Other methods which may be used for identifying proteins in a signal transduction pathway of a cell may comprise for example a western blot overlay method which method is well known to those skilled in the art. Cell extracts are run on SDS-gels to separate out protein and subsequently blotted onto a nylon membrane. These membranes may then be incubated, for example in a medium containing UNC-53 with a biotin label thereon and any protein conjugates visualised

with a streptavidin-alkaline phosphatase conjugated antibody.

The present invention also advantageously provides a process for the preparation of binding antibodies which recognise proteins or fragments thereof involved in the rate and direction of cell migration or the control of cell shape, for the above methods. Preferably the antibody is monoclonal antibody and more preferably monoclonal antibody 16-48-2.

The monoclonal antibody for binding to UNC-53 (or its functional equivalent) may be prepared by known techniques as described by Kohler R. and Milstein C., (1975) Nature 256, 495 to 497.

Another method which may be used to identify proteins involved in the signal transduction pathway involves investigating protein-protein interactions using the two-hybrid vector method. This method, which is well known to those skilled in the art, utilises the properties of the GAL4 protein in yeast. GAL4 is a transcriptional activator of galactose metabolism in yeast and has a separate domain for binding to activators upstream of the galactose metabolising genes as well as a protein binding domain. Nucleotide vectors may be constructed, one of which comprises the nucleotide residues encoding the DNA binding domain of GAL4. These binding domain residues may be fused to a known protein encoding sequence, such as for example unc-53. The other vector comprises the residues encoding the protein binding domain of GAL4. These residues are fused to residues encoding a test protein, preferably from the signal transduction pathway of C. elegans. Any interaction between the UNC-53 protein and the protein to be tested leads to transcriptional activation of a

reporter molecule in a GAL-4 transcription deficient yeast cell into which the vectors have been transformed. Preferably, a reporter molecule such as ß-galactosidase is activated upon restoration of transcription of the yeast galactose metabolism genes. This method enables any interactions between proteins involved in the signal transduction pathway to be investigated.

Any proteins identified in the signal transduction pathway of the cell, which may be for example a mammalian cell, may also be included in a pharmaceutical composition together with a carrier, diluent or excipient therefor.

The present invention also provides a process for producing an UNC-53 protein of C. elegans or a functional equivalent, fragment, or derivative of the protein, which process comprises culturing the cells transformed or transfected with a cDNA expression vector having any of the cDNA sequences according to the invention as described above, and recovering the expressed UNC-53 protein. The cell may advantageously be a bacterial, animal, insect or plant cell.

A particularly preferred process for producing UNC-53 protein comprises using insect cells. Accordingly, the invention provides a process for producing an UNC-53 protein of C. elegans or a functional equivalent, fragment, derivative or bioprecursor of the UNC-53 protein, which process comprises culturing an insect cell transfected with a recombinant Baculovirus vector, said vector comprising a nucleotide vector encoding the UNC-53 protein or a functional equivalent, fragment or bioprecursor thereof downstream of the Baculovirus polyhedrin promoter and recovering the expressed UNC-53 protein. Advantageously, this method produces large amounts of protein for recovery. The insect cell may be from for

example Spodoptera frugiperda or Drosophila Melanogester.

In accordance with the present invention, a defined nucleic acid sequence includes not only the identical nucleic acid but also any minor base variations from the natural nucleic acid sequence including in particular, substitutions in bases which result in a synonymous codon (a different codon specifying the same amino acid), due to the degenerate code in conservative amino acid substitution. The term "nucleic acid sequence" also includes the complimentary sequence to any single stranded sequence given which includes the definition above regarding base variations.

Furthermore, a defined protein, polypeptide or amino acid sequence according to the invention, includes not only the identical amino acid sequence but also minor amino acid variations from the natural amino acid sequence including conservative amino acid replacements (a replacement by an amino acid that is related in its side chains). Also included are amino acid sequences which vary from the natural amino acid but result in a polypeptide which is immunologically identical or similar to the polypeptide encoded by the naturally occurring sequence. Such polypeptides may be encoded by a corresponding nucleic acid sequence.

The invention may be more clearly understood from the following description with reference to the accompanying drawings and photographs, in which

Fig. 1 shows one strand of the C. elegans unc-53 mRNA translated into DNA (U to T) (5073 bases) which corresponds to the 8A clone variant encoding the corresponding 8A protein shown in Figure 3. Designations "TB" are positions onto which SL1 transplices have been identified at the 5' end of the sequence. Different mRNAs which differ in their 5'

end therefore exist. Potential start methionines are double underlined (M). Restriction endonuclease sites are indicated. A region of 8 sequential A bases at positions 4594 to 4601 is underlined. This region differs from the corresponding region of the known sequence in the database (F45E10.1) by having 8 rather than 7 A'denine (A) bases resulting in a frame shift (see Fig 15) and corresponds to the 7A form of the protein. The nucleic acid sequence from the database is also included in the nucleic acid sequences of the present application for reference only.

Fig. 2 shows a comparison of the sequences of the 7A and 8A clones of Figure 1.

Fig. 3 shows the predicted C. elegans amino acid UNC-53 sequence corresponding to the nucleic acid sequence of the 8A clone shown numbered from 1 to 1528. Again, potential start methionines are double underlined (M). Designations "tb" are regions for PCR clones to identify PCR products. Other regions of interest are identified. The region indicated as S4 is part of a lambda clone - 16.8 kb of the UNC-53 nucleic acid. This sequence, when translated is part only of the UNC-53 protein. Yet, injection of this part gives transformation rescue in organisms, i.e. providing additional evidence for the existence of shorter forms of the protein.

Fig. 4 shows the predicted C. elegans amino acid sequences of Figure 3 in the three letter code for indicating amino acids.

Fig. 5 shows the predicted C. elegans amino acid sequence UNC-53 sequence corresponding to the nucleic acid sequence of the 7A clone of Figure 2 shown numbered from 1 to 1583.

Fig. 6 shows the amino acid sequence of Figure 5 in the corresponding three letter code format for indicating amino acids.

Fig. 7 shows sequences of low complexity of the amino acid sequence of the corresponding nucleic acid sequence of the 8A clone of Fig. 3 identified with the filter and SEG algorithms of the BLAST sequence homology package. Regions of low complexity are indicated by "X" for the first copy of the sequence and by underlined amino acids for the second copy.

Fig. 8 shows, schematically, the known branches of the highly conserved Receptor Tyrosine Kinase/GRB2 signal transduction pathway including UNC-53.

Fig. 9 shows, schematically, the differences in cells with increased and decreased UNC-53 expression from the wild type.

Fig. 10 is a graph of the effect of anterior-posterior signal strength on growth cone extension rate of *C. elegans* organisms, with increased and decreased UNC-53 expression from the wild type. This graph translates the observation that UNC-53 acts in a dosage-dependent way to direct the rate of extension in the anterior/posterior axis into a model. The signal received e.g. (egl-15) is an RTK mediated signal which is postulated to be received by UNC-53 and which results in extension in the anterior/posterior axis. The graph shows an allelic series of organisms with a graded reduction in extension from increased UNC-53 expression down through wild type to a reduced UNC-53 expression. The prediction is thus: for the same level of RTK mediated signal the increased/decreased growth in the anterior/posterior axis depends on the level of expression of UNC-53 in any organism. The graph also reflects the prediction that for organisms with a particular level of UNC-53 overexpression there is no requirement for a signal before growth cone extension occurs. This extension is likely to be in a random direction or influenced by alternative factors.

Fig. 11 shows constructs of unc-53 nucleic acid including identified functional domains .

Fig. 12 shows 5′ amino terminus of the cDNA encoding from the first methionine amino acid through the actin binding protein homology domain (amino acids 1-133 from Fig. 1) and oligonucleotides designated oligo BG01, BG02 and BG03 (amplification strategies of amino terminus of the unc-53 cDNA). Combinations of oligo BG02 with either oligo BG02 or BG03 were used to amplify the 5′ terminus of the cDNA from the first methionine through the actin binding protein homology domain (amino acids 1-133). All of the oligonucleotides are underlined and sequences identical to the cDNA are shown in upper-case. In addition to unc-53 sequence, oligo BG02 contains a stop codon and the recognition sequence for BamHI endonuclease. Oligo BG01 has engineered EcoRI and NdeI recognition sites for inclusion in bacterial expression vectors. Both constructs remove the 5′ untranslated region of unc-53 and oligo BG03 contains a NotI cleavage site. Oligo BG03 has an improved ribosome binding site similar to mammalian ribosome binding sites. Use of BG03 in PCR thus results in constructs optimised for mammalian expression.

Figure 13 shows, schematically, constructs of the plasmids pTB109, pTB110, pTB111 and pTB112.

Fig. 14(a) shows a summary of transcript starts at the 5′ end of the unc-53 gene. Different identified transcript starts and corresponding in-frame ATG-codons are marked. Tab2 is the oligo from within cDNA M5 which was used in RT PCR experiment to identify/isolate the 5′ ends of different UNC-53 mRNAs.

Figure 14(b) shows the location of the different transcript starts on the genomic DNA and the position of the S4 Lambda clone with respect to genomic DNA.

Figure 14(c) shows the sequence near the 5′ and 3′ ends of the lambda S4 clone, identifying its composition corresponding to the 5′ and at position 2260 of comid COGHIO and the 3′ end of F45R10 at position 3287.

Fig. 15 shows the alignment of UNC-53 protein with the carboxytermini of the α-actinin and β-spectrin family (QY is UNC-53).

Fig 16 shows the predicted actin binding sites of UNC-53. The comparison shows internal LKK repeats.

Fig. 17 shows the alignment of the candidate SH3 binding sites in UNC-53 with known SH3 sites of other named proteins. Proteins at positions 4 and 7 are critical for binding into SH3 pockets.

Fig. 18 shows the alignment of the predicted amino acid sequences from F45E10.1 (available in public database) with UNC-53. The different identified amino acid is shown at position 1186. The frameshift which results in the different amino acid sequence from position 1513 is a result of the different number of adenine bases in the nucleic acid sequence (see Fig. 1).

Fig. 19 is a series of photographs of C. elegans embryos (strain TB4Ex25 (Table 1) [UNC-53-UNC-54 construct]). The photographs show increased outgrowth in the anterior-posterior axis of body wall cells in the C. elegans embryos which overexpress UNC-53 (immunofluorescence with UNC-53 mab 16-48-2)
Individual photographs are as follows:

A: early embryo comma stage
B: 1.5 fold stage embryo
C: 3 fold stage embryo, first plane of focus
D: 3 fold stage embryo, second plane of focus
E: 3 fold stage, mosaic animal, 3-cells in a quadrant giving expression.

This demonstrates that immunofluorescence

provides a measure of the expression in the transgenic lines of UNC-53.

Fig. 20A is a photograph of C. elegans embryo containing DNA construct pTB110 (strain TBAIn76(table 1)). Shown is expression of UNC-53 following heat shock.

Fig. 20B and C are photographs of C. elegans embryos containing DNA construct pTB111 (strain TB1Ex6 (table 1)). Shown is transgenic expression of UNC-53 in mechano-sensory neurons.

Fig. 21 shows photographs of the following:

A: A wild-type UNC-53 L1 larva of genotype 4-25 (strain TB4Ex25) as in photographs 19B, C and D.

B: L1 larva of 4-25 with morphological defects associated with muscle abnormalities.

C: Lethal phenotype of 4-25.

D: L1 larva of 4-25 showing misshapen animal and muscle cells with increased extensions. Also shows constipation problems associated with abnormal muscle pattern.

E: L1 larva of the heat-shock line TBAIn76 (table 1) exhibiting morphological abnormalities following heat shock and recovery.

F: L1 larva of line TBAIn76 (table 1) showing morphological defects in the pharynx.

All Figs. 19, 20 and 21 are Normarski optics of live embryos.

Fig. 22 is a map of plasmid pTB110 (tables 1 and 2) a heat shock promoter fusion, indicating restriction endonuclease sites.

Fig. 23 is a map of plasmid pTB112 (tables 1 and 2) a muscle specific UNC-54 fusion, indicating restriction endonuclease sites.

Fig. 24 is a map of plasmid pTB54 (the 8A clone variant) (tables 1 and 2). In the construction of this plasmid the complete unc-53 cDNA (tb3M5) of the

8A variant, including 5' and 3' UTRs was cloned as a NotI-ApaI fragment into the mammalian expression vector pcDNA3 (Invitrogen).

Figure 25 is a map of plasmid pTB72 (the construct encoding the 7A clone variant of UNC-53 cDNA of Figure 2.

Figure 26 is nucleotide sequence of the plasmid map of Figure 25.

Figure 27 is a map of plasmid pTB73.

Figure 28 is a nucleotide sequence of plasmid pTB73 of Figure 27.

Figure 29 is a map of plasmid pCB50.

Figure 30 is a nucleotide sequence of plasmid pCB50 of Figure 29.

Figure 31 is a map of plasmid pCB51.

Figure 32 is a nucleotide sequence of the plasmid pCB51 of Figure 31.

Figure 33 is a map of plasmid ppCB55.

Figure 34 is a nucleotide sequence of plasmid pCB55 of Figure 33.

Figure 35A illustrates a flowchart of the actin co-sedimentation assay. Soluble UNC53 protein was incubated with monomeric G-actin in a buffer containing ATP. Polymerization of G-actin to F-actin was induced by increasing the salt concentration to 100 mM, F-actin protein complexes were collected by centrifugation and analyzed by SDS-PAGE and fluorography.

Figure 35(B) illustrates the concentration series of the actin co-sedimentation assay. The full length UNC-53 encoding cDNA (pTB72) was transcribed and translated *in vitro* and co-sedimented with F-actin at a starting G-actin concentrations ranging from 0 to 250 mg/ml. See methods for details. S=supernatant after airfuging. P=pellet after airfuging.

Figure 35(C) illustrates both the full length

(pTB72) and amino terminal deleted UNC53 (pTB73) protein co-sediment with F-actin. Starting G-actin concentration was 500 mg/ml. S=supernatant, P=pellet, R= starting *in vitro* reaction.

Figure 36(A) is a flowchart of a SEM-5 binding experiment. The truncated UNC53 cDNA (pTB50) was transcribed and translated *in vitro* and incubated with SEM5-GST sepharose or GST sepharose. After four washes, the remaining proteins bound to the matrix were analyzed by SDS-PAGE and fluorography.

Figure 36(B) illustrates an immunoprecipitation experiment of radioactively labelled UNC53 proteins from the TnT pTB50 reaction shows that monoclonal antibody 16-48-2 recognizes both the native (-SDS lanes) and denatured (+SDS) protein products *in vitro*. c=control reaction without anti-UNC53 monoclonal antibody 16-48-2. ab=reaction with monoclonal antibody 16-48-2. See methods for details.

Figure 36(C) illustrates the results of SEM-5-GST binding experiments outlined in (a). *In vitro* translated UNC53 protein were analyzed by SDS-PAGE and fluorography. See methods for details. sup=supernatant

Figure 36(D) illustrates a western blot overlay experiment of UNC-53 (construct pTB61) expressed in bacterial cells. Cell lysates were denatured in Laemmli buffer and the proteins separated by 5-25% gradient SDS-PAGE. The arrowhead indicates the presence of full length UNC-53 in the induced bacterial lysate. Additional gels were blotted to nylon membrane, incubated with biotinylated GST or biotinylated GST-GRB2 protein and bound protein complexes subsequently detected with a streptavidin-alkaline phosphatase conjugated antibody. See methods for details. U=uninduced bacterial cell lysate, I=induced bacterial cell lysate.

Figure 37 is a series of photographs of C. elegans which illustrates overexpression of UNC-53 in body muscle cells results in over-extension along the longitudinal axis. Transgenic C. elegans embryos carrying the construct pTB113 were analyzed for UNC-53 activity by immunohistochemistry with the 16-48-2 antibody. Starting from the photograph (a) of the top left panel of Figure 37.

(A) and (B) illustrate ectopic growth cone spikes (indicated by the arrowheads) are observed early in myogenesis in the comma stage embryo. (C) and (D) illustrate over-extension of muscle cells in the head region of a three fold embryo during outgrowth. (E) illustrates over-extension is clearly observed along the anterior-posterior axis (indicated by the arrowheads) of a late 3 fold embryo.

Figure 38 is a map of plasmid ptb113.

Figure 39 is a nucleotide sequence of the plasmid ptb113 of Figure 38.

Figure 40 illustrates neurite tree length and fraction positive cells enhancement in a transfected cell C9 compared to wild-type cells C0. Black bars indicate fraction positive cells whereas hatched bars indicate neurite tree length cells, as described in example 8.

Figure 41 illustrates the results obtained following application of compound (I-(IH-pyrrol-2-ylmethyl)-2-piperidinone) to N4 transfected cells. The dark coloured bars indicate fraction positive C0 clones whereas the hatched bars of the chart indicate fraction positive C9 clones.

The following sequence listings are referred to in the specification.

Sequence 1D No 1: is a nucleic acid sequence

corresponding to the 7A nucleic acid sequence variant of Figure 2.

Sequence 1D No 2: is a nucleic acid sequence corresponding to the 8A nucleic acid sequence variant of figure 1.

Sequence 1D No 3: is an amino acid sequence corresponding to the amino acid sequence of the 8A variant of figure 3.

Sequence 1D No 4: is an amino acid sequence corresponding to the amino acid sequence of the 7A variant of figure 2.

Sequence 1D No 5: is an amino acid corresponding to the amino acid sequence shown in figure 7.

Sequence 1D No 6: is a nucleic acid sequence of the oligo BGO3 sequence of figure 12.

Sequence 1D No 7: nucleic acid sequence of the oligo BG01 sequence of figure 12.

Sequence 1D No 8: is a nucleic acid sequence of the oligo BG02 sequence of figure 12.

Sequence 1D No 9: is an amino acid sequence corresponding to the amino acid UNC-53(a) sequence shown in figure 17.

Sequence ID No 10: is an amino acid sequence corresponding to amino acid sequence of sequence (b) of UNC-53 shown in figure 17.

Sequence ID No 11: is an amino acid sequence

corresponding to the sequence (c) of an SOS shown in figure 17.

Sequence ID No 12: is an amino acid sequence corresponding to the sequence (d) of an SOS shown in figure 17.

Sequence ID No 13: is an amino acid sequence corresponding to the sequence (d) of an SOS shown in figure 17.

Sequence ID No 14: is an amino acid sequence corresponding to the sequence (f) of SOS 1359 shown in figure 17.

Sequence ID No 15: is an amino acid sequence corresponding to the sequence (g) of SOS 1377 shown in figure 17.

Sequence ID No 16: is an amino acid sequence corresponding to the sequence (h) of Dynamin shown in figure 17.

Sequence ID No 17: is an amino acid sequence corresponding to the sequence (i) of dynamin shown in figure 17.

Sequence ID No 18: is an amino acid sequence corresponding to the sequence (j) of PI3K p85 shown in figure 17.

Sequence ID No 19: is an amino acid sequence corresponding to the sequence (k) of P13k p85 shown in figure 17.

Sequence ID NO 20: is an amino acid sequence

corresponding to the sequence (l) of AFAP-110 shown in figure 17.

Sequence No 21: is an amino acid sequence corresponding to the sequence (m) of AFAP-110 shown in figure 17.

Sequence No 22: is an amino acid sequence corresponding to the sequence (n) of 3BP-1 shown in figure 17.

Sequence ID No 23: is an amino acid sequence corresponding to the sequence (o) of 3BP-1 shown in figure 17.

Sequence ID No 24: is an amino acid sequence which corresponds to the amino acid sequence from positions 106 to 133 of UNC-53 shown in figure 16.

Sequence ID No 25: is an amino acid sequence which corresponds to the amino acid sequence from positions 1093 to 1120 of UNC-53 shown in figure 16.

Sequence ID No 26: is a nucleotide sequence corresponding to the nucleotide sequence of ptB72 shown in figure 26.

Sequence ID No 27: is a nucleotide sequence corresponding to the nucleotide sequence of ptB73 shown in figure 28.

Sequence ID No 28: is a nucleotide sequence corresponding to the nucleotide sequence of pCB50 shown in figure 30.

Sequence ID No 29: is a nucleotide sequence

corresponding to the nucleotide sequence of pCB51 shown in figure 32.

Sequence ID No 30: is a nucleotide sequence corresponding to the sequence of pCB55 shown in figure 34.

Sequence ID No 31: is a nucleotide sequence corresponding to the nucleotide sequence of ptb113 shown in figure 39.

Sequence ID No 32: is an amino acid sequence corresponding to the amino acid sequence as numbered from amino acid 1 to 110 of the sequence figure 3.

Sequence ID No 33: is an amino acid sequence corresponding to the sequence as numbered from amino acid sequence 114 to 133 of the sequence of figure 3.

Sequence ID No 34: is an amino acid sequence corresponding to the sequence as numbered from amino acid sequence 487 to 495 of the sequence of figure 3.

Sequence ID No 35: is an amino acid sequence corresponding to the sequence as numbered from amino acid sequence 537 to 545 of the sequence of figure 3.

Sequence ID No 36: is an amino acid sequence corresponding to the sequence as numbered from amino acid sequence 1032 to 1037 of the sequence of figure 3.

Sequence ID No 37: is an amino acid sequence corresponding to the sequence as numbered from amino acid sequence 1097 to 1116 of the sequence of figure 3.

Sequence ID No 38: is an amino acid sequenc ecorresponding to the sequence as numbered from amino acid sequence 1300 to 1307 of the sequence shown in figure 3.

Sequence ID No 39: is an amino acid sequence corresponding to the amino acid sequence (a) of ∝-actinin (aact) shown in figure 15.

Sequence ID No 40: is an amino acid sequence corresponding to the amino acid sequence (b) of unc-53 shown in figure 15.

Sequence ID No 41: is an amino acid sequence corresponding to the amino acid sequence (c) of β-spectrin (spectrin) shown in figure 15.

Sequence ID No 42: is an amino acid sequence corresponding to the amino acid sequence (d) of ∝actinin (aact) shown in figure 15.

Sequence ID No 43: is an amino acid sequence corresponding to the amino acid sequence (e) of UNC-53 shown in figure 15.

Sequence ID No 44: is a amino acid sequence corresponding to the amino acid sequence (f) of β-spectrin (spectrin) shown in figure 15.

Sequence ID No 45: is an amino acid sequence corresponding to the amino acid sequence (g) of ∝-actinin shown in figure 15.

Sequence ID No 46: is an amino acid sequence corresponding to the amino acid sequence (h) of UNC-53 shown in figure 15.

Sequence ID No 47: is an amino acid sequence corresponding to the amino acid sequence (I) of β-spectrin shown in figure 15.

Sequence ID No 48: is a nucleotide sequence corresponding to the nucleotide sequence of S4 lambda clone shown in figure 14(c).

The inventors have established a set of processes particularly in C. elegans to select for inhibitors or enhancers of UNC-53. This screen is based on transgenic or mutant organisms or cells in which we have introduced a nucleic acid sequence encoding UNC-53 under the control of a specific promoter. In these organisms UNC-53 is over-stimulated as judged by increased extension of growth cones of muscle cells which over-express UNC-53 in C. elegans. This leads to a range of phenotypes in both embryonic and postembryonic development(from death to defective morphology and motility). These phenotypes can be scored with simple means at high throughput. Similar results can be obtained with heat shock specific lines. The basis of our test for inhibitors of the UNC-53 signal transduction pathway is reversal of this phenotype to an improved state of health.

We have constructed transgenic strains of C. elegans which over-express UNC-53 in body muscle. This results in increased extension of muscle cells and embryonic lethality (17 to 80% of transgenic organisms depending on the line used). These strains are used to directly screen for drugs which interfere with unc-53 genes, UNC-53 protein activity or any regulatory factor thereof to thereby suppress the background lethality.

Another process which may be used for selecting

inhibitors or enhancers of UNC-53 uses a constitutively active unc-53. This is achieved by mutating the nucleotide binding domain such that GTP or ATP is always bound or by covalently attaching SEM-5. In this strategy, transgenics (tissue cultured cell lines, or organisms such as nematodes) are generated which maintain unc-53 in a higher endogenous level of activity. Over-extension and subsequent lethality results in a greater proportion than that observed in the UNC-54/UNC-53 wild type lines. By screening for survivors after drug treatment, this assay specifically identifies inhibitors of downstream components in the signal transduction pathway.

Another process utilises an UNC-53 promoter. In this approach, an UNC-53 promoter is fused to a nucleic acid sequence encoding a reporter molecule, for example green fluorescent protein (GFP). Cells will glow when trans-acting factors bind to the promoter to activate transcription. By screening for cells which do not fluoresce, molecules which inhibit transcription of UNC-53 are identified.

The processes for selecting inhibitors and/or enhancers according to the invention are preferably carried out on whole animals. This can be done using a C. elegans system. The advantages of these tests include:

(1) The screening in a whole animal assay. C. elegans is a complex multicellular organism with a full nervous system, digestive system, etc. Its anatomy and development has been described in extreme detail. It is one of the best-characterised higher organisms at the genetic, molecular, developmental and cell biological level. Any observed changes to phenotype can be checked against this database.

(2) To study effects on rate and directionality of cell migration and the change of cell shape it is

important to leave the cells under study in a setting where they are surrounded by the in vivo interacting tissues, cells and substrates for cell migration etc. This can be done using whole *C. elegans* subjects. A situation has been created where the given pathway is over-stimulated leading to an easily scorable phenotype which can be reverted in any assay or process.

(3) The endpoint of the screen is the substantially increased health of the organism. This permits the exclusion of non-specific and toxic compounds.

(4) A complete and specific inhibition of UNC-53 in the transgenics will lead at the worst to the phenotype of an UNC-53 reduction or loss of function mutant which we have described, can recognise and have shown not to be essential for viability.

(5) The test can be adapted to make full use of the advantages of the *C. elegans* model system such as the possibility to conduct the test chronically over several generations and the possibility to conduct the test in different genetic backgrounds, e.g. RTK constitutive or defective.

(6) *C. elegans* exhibits a complex set of wild type, drug- and mutation-induced phenotypes such as changes in body shape, subtle changes in locomotion, mating behaviour, chemotaxis, pharyngeal pumping, egg laying behaviour, which can be used as part of a phenotype analysis or screen.

The results of *C. elegans* research described herein has provided important breakthroughs in biomedical research fields such as programmed cell death, neuronal guidance, the Receptor Tyrosine Kinase/RAS signal transduction pathway, integrin/cell adhesion receptor signalling, etc.,

The biochemical association of UNC-53 in the RTK signal transduction pathway enables identification of

genes or of biochemical pathways which are targets for pharmacologically or pharmaceutically active compounds and the development of high throughput and mode of action specific drug screens using wild type, mutant and transgenic animal strains including, in particular, C. elegans.

Thus pharmacological manipulation of the UNC-53 pathway is now possible on the following rationale:

We have scientific arguments to expect C. elegans UNC-53 to interact in vivo with the other components of RTK signal transduction pathways based on:

(1) The observation that C. elegans SEM-5 and GRB-2 are mutually exchangeable in vivo, combined with our observed in vitro binding of both GRB-2 and SEM-5 to UNC-53. Thus, C. elegans UNC-53 will be able to interact with the activated GRB-2/RTK receptor in mammalian cells.

(2) UNC-53 interacts with the rabbit actin-cytoskeleton

Expression of C. elegans UNC-53 in mammalian cell lines represents a shortcut to develop pharmacological assays and screens to target this pathway. We have shown that over-expression of the C. elegans UNC-53 in C. elegans myoblasts leads to over-extension of these cells in the anterior/posterior axis of the embryo and ultimate disorganisation of the muscle cell and myofilament pattern. (Over)-expression of C. elegans UNC-53 in a human cell line leads to a detectable change in phenotype, in particular increased motility of cells, increased outgrowth of neurons and morphological changes in the elongation and cytoskeletal morphology of differentiating myotubes.

The C. elegans unc-53 Open Reading Frame (ORF) (with and without optimised Kozak consensus sequence) of both 7A and 8A clone variants has been cloned between the CMV major intermediate early

promoter/enhancer and bovine growth hormone polyA signal sequence of expression vector pcDNA3 (Invitrogen). This vector is designed for high level stable and transient expression in most mammalian cells.

The following additional considerations require mention:

(1) Genetic analysis of reduction in UNC-53 function and ectopic expression experiments suggest that UNC-53 acts in a highly dosage-dependent manner. As is the case for RAS, increased expression may lead to lowering the threshold of RTK-signal required for a given response or may remove the requirement for an activating signal to obtain a phenotype response (Fig 10). In addition UNC-53 is an unusually low abundance protein in wild type C. elegans. It is therefore likely to be necessary or useful to control the temporal and quantitative expression of UNC-53 in the proposed assay conditions in all organisms or cells to be assayed. The already available or a further optimised expression cassette is then cloned in expression vectors with IPTG- inducible or tetracycline-repressible promoters. It is realised that both the Lac and Tet expression systems are leaky. Additional other repressible/inducible expression systems (e.g. Mx promoter) or weak mammalian promoters might be preferred.

(2) Over-expression of the endocytosis controlling protein dynamin leads to phenotypes which are not associated with dynamin function in the cell but which are thought to be due to sequestration of the GRB-2 pool in the cell (GRB-2 is an adaptor for a variety of signal transduction pathways). Such sequestration is unlikely to lead to "positive effects" on the activity of the cell such as is observed in the presently described assay system (increased cell process

extension or motility), see Fig 19. Based on the homology between UNC-53 and GTP-binding, we can also predict specific mutations in the nucleotide-binding pocket or the predicted effector region which should lead to loss of function. Sequence analysis of unc-53 alleles is instructive in determining which amino acids of UNC-53 are essential for function, e.g. as exemplified by the indication that an allele (n152) which has a differential effect on anterior versus posterior guidance has a deletion in a region of differential splicing. The differential splices of the C. elegans unc-53 gene encode different variants of the protein which independently affect posterior or anterior migration and/or cell specificity. One predicted exon in C. elegans unc-53 is indicated in Fig 1. It is conceivable that of two variants of the same protein one is inhibited or enhanced by a particular compound whereas the other is not (or to a lesser degree). Such a compound could then be used to control direction of migration or cell specificity by selective inhibition or enhancement.

(3) To develop pharmacological screens for inhibitors of a biochemical pathway a "gain of function" phenotype has been invented which can be expected to revert to wild type in the presence of specific inhibitors. Overexpression of UNC-53 in C. elegans myoblasts already leads to lethal subviable muscle phenotypes which can be easily scored with high throughput or a scorable heat shock inducible phenotype (Fig 21). They may form the basis for a pharmacological screen for inhibitors. A similar screen is obtained for over-expressing UNC-53 in mammalian cells. An alternative strategy is based on the homology to GTP binding proteins, RAS and dynamin and NTPases. We can introduce amino-acid changes in the nucleotide binding pocket which are

predicted/expected to lead to a constitutively activated or inactivated UNC-53. Similar changes are based on homologies with SOS, dynamin or ATP/GTP binding proteins from homology tables.

(4) Correct expression of UNC-53 in each cell line may be assessed by immunofluorescence and western blot analysis with the monoclonal antibody (mab) designated as 16-48-2.

The inventors have thus expressed and stably integrate the expression constructs in the neuronal, myoblast and 3T3 cell lines.

These cell lines are primarily used to:

- Assess the effect of UNC-53 expression on the morphology, motility, metastatic potential and growth cone extension of the cell lines.
- Produce protein and mRNA
- Screen for pharmacological compounds inhibiting observed UNC-53 mediated phenotypes
- Analyse signal transduction pathways associated with UNC-53 activation (for example, phosphorylation,)
- Immunofluorescence studies with mab 16-48-2 to assess changes in subcellular localisation following growth factor treatment.

Thus, the present invention provides for the identification of compounds which inhibit or enhance the UNC-53 signal transduction pathway. Such compounds can be used in the control of cell directional migration, motility and differentiation. These compounds are useful in the treatment of oncogenesis, psoriasis, neuronal degeneration and cell migration (metastasis).

The present invention also provides the ability to identify nucleic acid sequences and proteins which are involved in the UNC-53 pathway in C. elegans. Such nucleic acid sequences and proteins may be UNC-53 equivalents, members of an UNC-53 pathway or may be

nucleic acid sequences or proteins which interact in the UNC-53 pathway, for example as demonstrated by the GRB-2/SEM-5 proteins. This knowledge of the UNC-53 pathway in C. elegans can be established as can factors which influence the functioning of the pathway, for example, factors/ proteins which feed into the pathway or are of a parallel pathway which at least, in vitro, compensates for steps in an UNC-53 pathway.

The identification of other components in the UNC-53 signal transduction pathway:

(1) help to determine the interaction of UNC-53 with known signal transduction pathways (RAC-, RHO-, cdc42-RAS-pathway exchange factors, downstream or regulating kinases)

(2) identify the new interacting proteins which may constitute additional potential pharmacological targets.

(3) may assign functions to the more than 1000 amino acids of UNC-53 which have no homology to known proteins.

Accordingly, proteins which cross-react with anti-C. elegans UNC-53 protein antibodies can be isolated. The basic experiment protocol for purifying antigen-antibody complexes is described in Example 11. This system can also be used to identify factors which interact with proteins which bind to anti-UNC-53 C. elegans antibodies.

The following tissue sources may be used for immuno-precipitation:

(1) Mammalian cells which exhibit a phenotype after transfection with unc-53 indicating that it interacts with vertebrate components of its signal transduction pathway.

(2) UNC-53 protein may be too low abundance to make affinity purification from wild type C. elegans

feasible. The inventors have affinity-purified UNC-53 from already constructed transgenic C. elegans lines which express UNC-53 under control of the hsp-16 promoter and/or the myosin promoter. These experiments in C. elegans are justified because with the vast amount of sequence information (genomic and cDNA) available, one has a good chance of identifying the corresponding genes in the databases with a minimum of peptide sequence.

Several types of proteins may be expected to co-purify with UNC-53, including GRB-2 and other proteins with SH3 domains of the Grb2 class or phosphorylation sites, RTK-receptors, subunits of an UNC-53 homo-heterodimer complex, downstream regulating kinases or proteins from the microfilament cytoskeleton.

This co-immuno-precipitation approach can also be used to dissect the order of events in this signal transduction pathway. For example: UNC-53 immuno-purified after stimulation of mammalian cell-lines with growth factors and pharmacological agents can also be assayed with respect to its state of phosphorylation, or complex formation with interacting proteins.

Proteins interacting with specific UNC-53 domains are identified using a yeast two-hybrid system, whereby two sets of hybrid proteins are used to assay for functional restoration of the GAL4 transcriptional activator: the first consisting of a GAL4 activation domain/UNC-53 structural domain of unknown function, the second derived from a cDNA library cloned into an expression vector to generate a library of hybrid proteins containing a GAL4 DNA binding domain. The yeast two-hybrid system is well know in the art.

A set of unc-53-fusion constructs can be constructed, including a fusion to

(1) the full length protein,

(2) the carboxyterminal domain (from second actin binding domain to the ATP/GTP binding domain),
(3) The aminoterminus (predicted cortical localisation domain up to the SH3 binding sites),
(4) a variety of overlapping constructs within the central domain of 1000 amino acids to which no function can as yet be assigned.

These are tested in yeast to exclude those which lead to activation of the reporter gene in the absence of the cDNA-activator fusion. cDNA libraries were transformed into these reporter strains and positive clones identified. (In this strategy, screening of multiple libraries requires very little effort (transformation followed by plating on selective and indicator medium)).

A preferred cDNA library is from cell lines in which a phenotypic change is observed following UNC-53 expression such as mouse N4 neuroblastoma cells or MCF-7 breast carcinoma cells. The yeast two hybrid system can identify interacting proteins or "sections" of nucleic acid which may not be translated in vivo but which may inhibit UNC-53.

Candidate positives are tested for the fusion-protein dependence of the reporter gene activation. The cDNA insert in remaining positive clones is sequenced. The obtained sequence is screened through the databases, which provides, especially in the case of C. elegans clones, significant extra sequence.

Another system also exists for the identification of proteins which bind or modify UNC-53. An UNC-53 protein is bound by conventional techniques to a column. A sample to be tested is then passed over the column. This sample may be fractions from cells from C.elegans, mammals or any other organism. These sample fractions may have been incubated with ^{32}ATP. In this course the "reaction" of the labelled fraction

with UNC-53 can be determined. If the UNC-53 on the column becomes ^{32}P phosphorylated then this indicates that the sample fraction contains an UNC-53 modifying protein. Alternatively a constituent of the sample may bind to the UNC-53 and remain bound therewith on the column. The retention of any fraction of the sample on the column and the identification of the fraction can easily be determined by techniques known in the art.

Example 9 describes the identification of sensitive, dependant or resistant mutations as direct tools for the development of screens for compounds with similar or antagonistic activities. Both resistant and sensitising mutations may have a phenotype in the absence of the compound and no or a different phenotype in the presence of the compound. This permits the introduction of action-specificity in the screens.

High throughput screens are a basic feature of <u>C. elegans</u> genetic methodology. Non-complementation screens for new alleles in a locus require setting up of up to 8000 separate worm populations starting from one hand-picked individual each. This is done in 24 well plates or small Petri-plates. These are subsequently (after 1 or 2 generations) visually screened for a complex behavioural phenotype. For pharmacological screens where populations can be started from multiple individuals pipetted from a pool of synchronised eggs, high throughput screens can also be developed. If the endpoint of the assay can be scored in liquid, populations can be set up in microtitreplates. If the end-point is linked to a reporter gene (e.g. β-galactosidase activity) ELISA type colour-metric assays can be used to score the end-point. <u>C. elegans</u> can also be introduced into soils, exposed to compounds and subsequently recovered

and assayed. Such endpoints are used in the heat-shock assay developed by Stressgen (Stringham & Candido (1994), Environ. Toxicology and Chemistry, 13(8), 1211-1220).

Gain of function mutants of *C. elegans* or transgenic *C. elegans* in which a pathway of interest has been over- or constitutively activated, causing a dominant phenotype which can be used to develop specific screens for inhibitors.

Transgenic lines expressing UNC-53 ectopically under the *C. elegans* heat-shock (hsp-16) promoter, and body wall muscle (unc-54) promoter have been constructed. These lines lead to dominant phenotypes in development and are used directly to screen a spectrum of compounds. Where necessary or deemed useful endogenous *C. elegans* genes can be replaced by or complemented with human signal transduction pathways.

DEPOSITED CELL LINES AND PLASMIDS

STRAIN NAME	DATE OF DEPOSIT	LMBP ACCESSION NUMBER
pTB54 Plasmid	22 MAY 1995	3296
pTB112 Plasmid	22 MAY 1995	3295
pTB72	22 MAY 1996	3486
TB4EX25 Cell Line	22 MAY 1995	1384 CB
TBAIn76 Cell Line	22 MAY 1995	1385 CB
HYBRIDOMA Cell Line	22 MAY 1995	1383 CB
MCF-7 TRANSFECTED BREAST CARCINOMA		

CELL LINE	24 MAY 1996	1550 CB
TRANSFECTED N4 NEUROBLASTOMA CELL LINE	24 MAY 1996	1549 CB
WILD TYPE MCF-7 BREAST CARCINOMA CELL LINE	24 MAY 1996	1551 CB

The above plasmids and cell-lines were deposited at the Belgian Coordinated Collections of Micro organisms (BCCM) at Laboratorium voor Moleculaire Biologie - Plasmidencollective (LMBP) B-9000, Ghent, Belgium, in accordance with the provisions of the Budapest Treaty of 28 April 1977.

The present invention will now be described with reference to the following Examples.

Examples

Example 1 - Molecular Characterisation of unc-53 gene in C. elegans

Screen for muscle pattern mutants :

C. elegans has two sets of muscles which are suitable to study this problem, the body wall muscles and the sex muscles. The sex muscles are a set of 16 muscle cells (4 muscle types) in the hermaphrodite and 41 cells in the male (10 muscle types) with distinct attachments points on the hypodermis and gonads. The sex muscles develop postembryonically and are not required for viability. The body wall muscles are arranged longitudinally (roughly 2 cells abreast) into four quadrants. At birth there are 81 cells. In postembryonic development, extra muscles interdigitate with these bringing the total number of body wall

muscles in the hermaphrodite to 95. Head, neck and body muscles can be distinguished within these rows on the basis of their innervation and patterning within the rows.

We have screened 4800 haploid genomes using Nomarski and polarized microscopy for mutants with specific attachment or pattern defects in a subset of the male sex muscles but with wild type body wall muscle pattern and myofilament organization, wild type movement and wild type male bursa anatomy (a sensitive indicator of wild type morphogenesis). Amongst the 21 identified mutants we selected for further study those with specific phenotypes in both the male and hermaphrodite sex muscles. As these muscles lie in different regions of the animals this was thought to reduce the chance that the male tail phenotype is a pleiotropic consequence of changes in regional identity of the tail or defects in male tail hypodermal lineage or morphogenesis.

<u>Muscle phenotype of e2432.</u>

Mutant e2432 was isolated on the basis of its phenotype in the male spicule retractor muscles, a pair of bilaterally symmetrical muscles which attach anteriorly to the body wall and posteriorly to the base of the spicules. The spicule retractors of mutant e2432 are shorter than wild type. Their attachment to the spicules is wild type, but their attachment point to the body wall is shifted posteriorly. The spicule protractors sometimes extend processes onto the attachment point of the spicule retractors on the hypodermis, suggesting the defect is not in these attachment points, but rather in the extension of the muscles towards that point. The diagonal muscles are in most specimens wild type but they are occasionally not parallel to one another or are have a dorsal

attachment point that is more ventrally positioned than in wild tye. e2432 males have a nicely shaped fan with the normal pattern of rays, suggesting that the sex muscle defect is not pleiotropic due to defects in the hypodermis.

e2432 hermaphrodites have a reduced ability to lay eggs which is variable from animal to animal. This is due to a muscle pattern defect in the vulval sex muscles. The uterine muscles, 8 muscle cells which circle the hermaphrodite uterus, are wild type in e2432. The vulval muscles are a set of 4 pairs of cells arranged symmetrically in a cross-pattern around the vulval slit. Each pair consists of one vm1 and one vm2 muscle cell. The vm2 muscles attach to the junction between uterus and vulva and extend anteriorly to attach to the hypodermis in between two muscle cells of the ventral body wall muscle quadrant. In e2432 these muscles are shorter than in wild type small. In e2432 they can only be visualized by laser confocal microscopy (after FITC-phalloidin staining of the myofilaments). This showed that they attached to the uterus as in wild type, but that their attachment to the body wall is ectopic (in a random position lateral of the vulva, usually on the ventral edge of the muscle row). In e2432 vm2 myofilaments are oriented more dorsoventrally than in wild type (where their orientation is essentially in the longitudinal axis of the animal). This phenotype is not due to a defect in the attachment point on the epidermis to which these cells should attach in wild type, since we frequently observe that the vm1 sex muscles make an apparently wild type attachment to this unoccupied attachment point.

In wild type hermaphrodites, the vm1 muscle cells attach close to the junction between epidermis and vulva and in the adult extend dorsally and anteriorly

(under an angle of 45-50 degrees with respect of the vulval slit) to attach to the hypodermis at the dorsal edge of the ventral body wall muscle quadrants. In e2432 the attachment of the vm1 muscles to the vulva is wild type. With their other end they attach, like wild type vm1 cells, along the dorsal of the edge of the ventral body wall muscles. However the angle between the vulval slit and the myofilaments of the vm1 sex muscles is reduced (less than 45 degrees) so that their dorsal attachment point is closer to the vulva than in wild type. The forces acting on the vulva can be separated in an antero-posterior and a dorsal vector. In e2432, the antero-posterior vector of both the vm1 and vm2 muscle is significantly reduced, leading to a reduced ability to open the vulva upon contraction. Studies in which vulval muscles were ablated individually or in groups suggested that 2 vulval muscle cells of wild type orientation are sufficient for wild type function.

Adult C. elegans hermaphrodites have 95 body wall muscle cells arranged longitudinally (roughly 2 cells abreast) into four quadrants. In wild type cells these cells are spindle shaped.

e2432 adults have body wall muscles with a wild type muscle cell and myofilament pattern, except that cells with interdigitating tips occur more frequently than in wild type. Like the unc-53 phenotype in the male and hermaphrodite sex muscles, this body wall muscle defect, which can also be observed in other guidance and attachment mutants like unc-6 and mups, can also be attributed to a reduced ability to extend "growth cones" otherwise referred to as cell processes in the anterior-posterior axis of the animal.

Position on the genetic map :

e2432 was mapped to the left arm of chromosome II

and was found not to complement unc-53(e404). The unc-53 locus was originally identified by Brenner (1974), Genetics, <u>77</u>, 71-94 as one of the uncoordinated mutants but has received only sporadic attention in general phenotypic surveys of the UNC-collection (Hedgecock <u>et al</u> (1987), Development, <u>100</u>, 365-382 and Siddiqui (1990), Neurosci. Res. (Suppl) <u>13</u>, 171-190, in a genome wide screen for egg laying defective mutants (Trent and Horvitz (1983), Genetics, <u>104</u>, 619-647) and using e2432 as a tool to study the effect of body shape on the pattern of neuronal processes (Hekimi and Kershaw (1993), J. Neuroscience, <u>13(10)</u> 4254-4271). We initiated a detailed genetic and phenotypic analysis of this locus using the existing available alleles which various colleagues isolated in different screens : The canonical unc-53 allele e404, a strong UNC was isolated by Sydney Brenner. Alleles n152, n166 and n1199 have been obtained in screens for egg laying defective mutants. Alleles NJ234 and NJ222 were isolated by Ed Hedgecock in a screen defective in excretory canal outgrowth. As these screens were aimed at isolating viable fertile alleles, we isolated additional alleles by pre-complementation screens designed to yield loss of function alleles irrespective of their phenotype. e2432/mnDf90 hermaphrodites are egl, weak unc's with a slightly stronger phenotype than e2432. Matings were set up on 3 cm petri dishes between 2 to 3 unc-53(e2432) sqt-1(sc13) /+ males and 2 e2431ts or dpy-6(e14) hermaphrodites mutagenized with EMS in the L4 stage (Brenner, 1974) , Genetics, <u>77</u> 71-94. The F1 egl, unc-53 like hermaphrodites, which may be unc-53(e2432) sqt-1(sc13)/unc-53(new) were cloned on petri dishes and their offspring examined for the segregation of new unc-53 alleles. In two screens, two unc-53 alleles, 5 and 8 were isolated in an estimated 13000

F1 offspring, giving an approx. mutation rate 1/3250 mutagenized chromosomes. Sqt-1(sc13), an allele of sqt-1 that confers a roller phenotype was included because it is closely linked to unc-53 (0.2 m.u.) and marks the original allele e2432. e2431ts, an X-linked ts larval lethal with a mup phenotype was included to eliminate F1 hermaphrodites arising from selfing and F1 males which can mate. In the second screen dpy-6(e14) was included to prevent F1 males from mating with F1 hermaphrodites.

All unc-53 alleles used in this study fail to complement to e2432. Complementation was tested by mating unc-53(e2432) sqt-1(sc13)/+ males to hermaphrodites of the respective alleles. The male sex muscle phenotype described above for e2432 was found to be the only 100% penetrant phenotype in the unc-53 locus (see below) and was the primary phenotype used in complementation tests. Each of these alleles was also complemented to mnDf90 by mating unc-4 mnDf90/mnC1 males to unc-53 homozygotes and temporary unc-53/unc-4 mnDf90 lines were established to evaluate the phenotype. The male and hermaphrodite phenotypes of all alleles over deficiency is identical or slightly, but not substantially stronger than that of the homozygous lines (which is not unusual for a large deficiency).

S. Brenner mapped unc-53 to 2.9 +/- 0.7 map units from dpy-10 (chromosome II). We refined this map position by mapping unc-53 with respect to different deficiencies in the region and doing three factor crosses between unc-4 and sqt-1, a 1.5 map unit interval. Unc-53(e2432)/+ males were mated in unc-4 sqt-1 hermaphrodites. Non-rolling F1 offspring were cloned on petriplates and their broods screened for the segregation of unc-53(e2432). Unc-4 non sqt-1 and sqt-1 non unc-4 hermaphrodites were picked from those

plates and cloned on petriplates. 6 out of 42 sqt-1 non unc-4 recombinants segregated unc-53 and 3 out of 18 unc-4 non sqt-1 recombinants did not segregate unc-53. This yields a relative position of unc-4 / 51 / unc-53 / 9 / sqt-1. Or a calculated map position for unc-53 on chromosome II, 0.23 map units left of sqt-1.

Unc-53(e2432) was mapped relative to three deficiencies in the region mnDf90 mnDf87 and mnDf77 by mating e2432/+ males to unc-4 Dfx/mnC1 hermaphrodites and scoring for males and hermaphrodites with the unc-53 phenotype in the F1. The experiment was also performed by mating unc-4 mnDfx/mnC1 males to homozygous unc-53. mnDf87 and mnDf90 do not complement unc-53 while mnDf77 complements unc-53. Ooc-3, the only other gene on the genetic map in the region, was found to complement unc-53 in identical crosses between e2432 and unc-4 ooc-3/mnC1. Further mapping of unc-53 relative to RFLPs between wt strains in the region and the molecular cloning confirmed the map position of unc-53 (see below).

Molecular characterization :

We started cloning the unc-53 locus because the study and interpretation of the unc-53 phenotype and the different mutants in the locus would be greatly facilitated by having information on and probes for the unc-53 mRNA and gene product.

At the time we initiated cloning of unc-53, a contig extending between unc-4 and sqt-1 (approx. 1500 kb) had been identified by A. Coulson and J. Sulston (C. elegans genome project LMB Cambridge), with no clone markers in between. To correlate the genetic map with the physical map in this region we positioned cosmids of this contig relative to the deficiencies mnDf77, mnD87 and mnDf90 by comparing band intensities of Southern blots of mnDfx/mnC1 strains probed with

cosmids throughout the region. Cosmid KO2F7 is deleted in mnDf90 but not deleted in mnDf87 an mnDf77 thus identifying a leftmost location for unc-53. Cosmids W10G4, TO8D11 and F33G3 are in the unc-53 region (not deleted in mnDf77 but deleted in mnDf87 and mnDf90). Cosmid KO4H9 is deleted in mnDf77 and identifies a rightmost location for the gene. The distance between KO2F7 and KO4H9 is approx. 10 cosmids.

To narrow down the position of unc-53 further we looked for restriction fragment length polymorphisms between wild type strains in this interval and identified N2/RC301 RFLPs in cosmids W10G4, F40F8 and F22G3. We mapped these using three factor crosses with the strains unc-53 sqt-1/RC301 and unc-4 unc-53/RC301. We mapped F22G3 and F40F8 between unc-53 and sqt-1 at the following relative distances :
unc-4 / 9 / W10G4 / *2* / unc-53 / *1* / F40F8 / 1 / F22G3 / sqt-1.

These data localize unc-53 in an interval of approx. 80kb in which more than 15 differently overlapping cosmids are available. Pools of cosmids were injected in unc-53(n152) gonads together with the rol-6 selectable marker. Transient roller lines were established and scored for rescue of the unc-53 phenotype. Cosmid T28D2 was found to rescue the backward movement egg laying phenotypes of allele n152 .

A genomic library of N2 in lambda 2001 was screened with T28D2 and flanking overlapping cosmids. These were assayed in pools and individually for transformation rescue. Lambda clone, S4 carrying a sixteen kb insert was shown to give some rescue activity. Using restriction fragments of S4 as a probe, cDNA clones M5 (3.8 kb) and M18 (1-2 kb) were

isolated from a Lamda MGU1 cDNA library. Both M18 and M5 contain an identical 3'-end as judged by restriction fragment analysis. Partial sequence analysis showed that M18 is shorter version of M5. Insert M5 was sequenced on both strands and was found not to be a poly-A tail at its 3'-end but appears not to full length at its 5'-end.

To find the 5' end of the unc-53 transcript we did nested PCR on L2 stage random primed cDNA, between antisense oligos tab2 and tab (43 bp away from the 5' end of cDNA M5) and an oligo to the SL1 trans-spliced leader sequence. This sequence is transspliced to the 5'-end of most <u>C. elegans</u> mRNAs. This yielded at least 6 classes of PCR-fragments which have been subcloned and sequenced. All contain the 43 bp between oligo tab2 and the 5' end of cDNA M5 (bp1281 to 1338). The longest PCR fragment (TB3) extends the sequence of cDNA M5 with 1280 bp. When added to the length of the cDNA M5, this unc-53 transcript which we constructed <u>in vitro</u> and named tb3-M5 would then be 5073 bp long (including some poly-A tail) and have a 1528 AA open reading frame. Recently a 5 kb cDNA, was identified in an embryonic cDNA library which has the TB3-5'-end (including part of the SL1), and the same 3'-end as M5, suggesting that TB3-M5 occurs <u>in vivo</u>. Similar PCR reactions in which the SL1 oligo was replaced by an SL2 transplice oligo gave no reaction products. Preliminary Northern blot analysis identifies a major 5.0 kb transcript and at least 2 smaller transcripts that are expressed in L2, L4 and adult worms. It needs to be examined whether the unc-53 5' ends reported here are made <u>in vivo</u> and encode different proteins or whether they represent PCR noise. The smaller PCR-fragments TB1b, TB16, TB1, TB6b and TB22 are "nested deletions" of clone TB3 with SL1's at their 5' end. The sequence of each is identical in the

regions of overlap. The shorter SL1 transspliced transcripts contain ATGs downstream of the SL1 addition sites at positions 466, 988 and 1324. Comparison to the sequence of genomic clones confirmed that the SL1s are spliced onto intron exon boundaries. However not all intron-exon boundaries receive SL1, suggesting that there is some specificity to this differential trans-splicing.

Recently the C. elegans sequencing consortium has sequenced cosmids F45E10. We mapped cDNA tb3-M5 onto these cosmids and found that unc-53 is an unusually large locus. It has 23 exons spread over more than 31 kb of genomic DNA.

The lambda clone S4 that rescues does not contain the first 430 bp of the unc-53 transcript. This suggests that the ORF between positions 63 and 430 is not essential for transformation rescue. This rescue may derive from expression of transcripts TB6b or TB22 or from "non-specific" initiation of transcription on the extrachromosomal arrays.

Additional confirmation that M5 was derived from the unc-53 transcription unit is provided by the observation that allele n152 has a 300 bp deletion, disrupting the sequence of cDNA M5 and leading to a large (possibly complete) reduction of UNC-53 protein in n152 embryos stained in immunofluorescence with an anti-unc-53 antibody (16-48-2). In addition, allele e2432 was found to carry a 3-4 kb insertion in this transcription unit.

Sequence homology :

Antibody staining :

The NdeI-EcoRI fragment of cDNA M5, the 47 kd fragment of UNC-53 encoded by the NdeI-EcoRI (position 3187 to 4458 (tb-M5 fig 3) protein sequence

fig 2) was subcloned in the T7 expression vector prk172 (yielding vector TB66 and expressed in E. coli. Inclusion bodies containing recombinant protein were purified, by processes known in the art solubilized in 8 M Urea and the recombinant protein purified over a DEAE column equilibrated in 8M urea. Purified protein was mixed with complete Freund's adjuvant and injected in a rabbit and 4 Lou rats. This was followed six weeks later by bi-weekly boosts with antigen mixed with incomplete adjuvant. All sera are active in western blotting at titers of 1:30,000 on Western blots of the 47 kd unc-53 fragment expressed in E.coli. With this western blotting assay, a rat-mouse hybridoma cell line was prepared producing a monoclonal antibody to UNC-53. Mab 16-48-2 has the following properties :

- protein G-binding
- binding activity on western blots of
(1) the 47 kd UNC-53 fragment expressed in E. coli, (pTB66)
(2) the 57 kd carboxyterminal fragment of UNC-53 expressed in E. coli (construct pTB65.)
(3) the full length TB3-M5 UNC-53 expressed in E. coli (construct pTB61) and mammalian cells (COS-cells; constructs pTB54 and 56).
- immunoprecipitation of native and SDS denatured full length TB3-M5 UNC-53 construct pTB50 expressed in vitro-transcription translation reactions in reticulocyte lysates.
- immuno-histochemistry in wild-type C. elegans fixed with methanol, acetone or paraformaldehyde and transgenic C. elegans expressing UNC-53 tb3-m5 pTB110, 111 or 112 in epidermis, neurones, gut and muscle.

Mab 16-48-2 fail to detect antigen of the correct size on Western blots of total worm proteins or worm proteins fractioned by progressive extraction with

detergents, urea and SDS.

Excretory canal phenotype :

The excretory canal of C. elegans is a large H-shaped cell. It's cell body is positioned ventrally at the level of the pharyngeal bulb and send out two processes dorsally. At the level of the lateral epidermis (seam) each of these bifurcates and extends anteriorly and posteriorly over the seam cells, until they extend over most of the whole body length. It has been reported that in unc-53 the posterior process of the excretory cell does not extend up to the V6/T seam-cell boundary (E. Hedgecock et al., (1987), Development, 100 365-382).

We have done an extensive characterization of this phenotype in all alleles listed, either by direct in vivo Nomarski microscopy or UL6 rol6d marked unc-53 strains which express LacZ in the epidermis and excretory cell (Hope(1991) Development 113(2) 399-408). In wild type the excretory cell processes are straight. In unc-53 the canal is often meandering from left to right over the seam before it arrests prematurely, as if it has lost directional cues in its migration. It never leaves the lateral epidermis seam. Both the anterior and posteriorward processes are affected.

In weak unc-53 alleles the posterior excretory canal processes arrest anywhere between the vulval region and the V6/T boundary. We noticed that in even the strongest alleles or in unc-53/Df heterozygotes the canal arrests unusually frequently at or close to the vulva and never substantially before the vulva . We therefore set out to test whether the gonad dependent attractive signal which attracts the sex myoblasts to the gonad also might attract the excretory canal in an unc-53 independent manner to the

vulval region. If this is the case we would expect that in a strong unc-53 mutant n152 in which the 2 somatic gonad cells (the source of the signal) have been ablated, the excretory canal migration would be fully arrested. As a control we ablated one germ cell and one somatic gonad cell (Z1 and Z2 or Z2 and Z4). Embryos were ablated in the comma to 2 fold stage and the position of the excretory canal scored double blind in hatched embryos. At the time of ablation, the canal may already have started growing out. At hatching, the endpoint of our experiment, the growth cone of the posterior canal process has reached just beyond the gonad. Although these are technically difficult laser ablations, the results show a substantial difference in excretory canal outgrowth between embryo with an ablated somatic gonad and control ablated embryos. In the experimental series the canal usually arrested a significant distance from the gonad or any other potentially damaged cells, suggesting the loss of a long range signal as described for the SM myoblast migration (Thomas et al (1990) and Stern (1991)). In the control series the excretory canal usually extended as far as unablated n152 and into region of the partially ablated gonad. This indicates that the premature arrest observed in the experimental series was not due to encountering a damaged region.

A gonad dependent and independent pathway were found to act redundantly in the posteriorard migration of the sex myoblasts. The data suggest that in wild type the migration of excretory cell growth cones is also guided by a gonad dependent and a gonad independent cue. In both cases the gonad dependent cue acts towards the gonad, but from opposite directions. However the gonad independent signal act anteriorward on the SM myoblasts and posteriorward on

the posterior excretory cell growth cones. Since single mutants in both the gonad dependent pathway (sem-5) and independent pathway (unc-53) have no excretory cell phenotype these pathways may be redundant in the trajectory up to the gonad. An analogous redundancy has been observed for the sex myoblast migration. In the trajectory between gonad and tail the gonad independent pathway acts in different directions on the SM cells versus the excretory cell. In the excretory cell it acts in both anteriorward and posteriorward migration. A simple explanation which is elaborated in detail below is that unc-53 (like sem-5) may act downstream of a variety of receptors interpreting different cues.

The previously described interaction between the gonad and the sex myoblasts was rationalizable as an interaction between cells due to become part of the same organ. The interaction between the excretory cell and the gonad we report here suggests that the gonad may have a more general role as organizer cell migrations in the embryo. We wish to point out that the described dependent and independent pathways are formal genetic concepts. It is for example possible that in unc-53 embryos or unc-53 embryos in which the gonad dependent pathway has been genetically or laser ablated, as yet to be identified, pathway defining growth cones are misplaced leading indirectly to defective sex myoblast, neuronal (PLM, see below) or excretory canal migration. The observed highly restricted expression of unc-53 is an additional indication of this possibility.

Sex muscle phenotype :

All unc-53 alleles exhibit the sex muscle phenotype described for e2432. We quantified phenotype

in eight alleles :

Young adults grown at 20°C were mounted for polarized light or Nomarski microscopy on 2% agarose pads containing 0.2% phenoxypropanol as described in Sulston and Horvitz (1977) Dev. Biol. 56,110-156 . The vm1 sex muscles were examined under polarized light with a 40x objective and a Brace Kohler compensator and photographed. In addition, adults were fixed, incubated with fitc-coupled phalloidin and mounted for fluorescence microscopy as described in Goh and Bogaert (1991) Dev. Biol. 56, 110-156. The angle between the longitudinal axis of the animal and the central bundle of myofilaments of the anterior and posterior vm1 was measured from the negatives with a protractor. As the vulva is a transverse slit at a right angle to the cylindrical body axis, the angle between the vm1 and the vulval slit can be measured independently of which side of the animal faces the observer.

Neuronal phenotype :

Unc-53 animals move poorly backwards when prodded but has good forward movement (Brenner (1974) Genetics 77 71-94). Various aspects of the neuronal phenotype of unc-53 has been reported in general phenotypic surveys of the UNC-collection (Brenner (1974) Genetics 77 71-94). : The posterior branch of the PDE neuron can be abnormal (Hedgecock et al. (1987) Development 100 365-382) and the mechanosensory PLMR & PLML neurons can have commissures into the ventral cord at a position much posterior than in the wild-type. There are also frequently multiple ventralward PLM commissures evenly spaced along the posterior half of the body (Siddiqui (1990) Neurosci. Res. (Suppl) 13 171-190), Hedgecock et al., (1987) Development 100 365-382).

Examples 2 to 5 - Biochemical Analysis of UNC-53

Example 2 - Immunoprecipitations of ^{35}S labelled unc-53 gene products.

The rat anti-UNC-53 monoclonal antibody, 16-48-2 (obtained from the hybridoma LMBP Accession no. 1383CB) elicited against a 47 kD fragment of the 3' end of UNC-53 from C. elegans was used to immunoprecipitate UNC-53 proteins. In this experiment, the full length unc-53 construct pTB50 (Fig. 11) was transcribed and translated in vitro in rabbit reticulocyte lysates. The resulting radioactively labelled ^{35}S unc-53 gene products were incubated with the monoclonal antibody under both denaturing (using SDS)and non-denaturing conditions, then incubated with protein G sepharose. The bound products were analysed by SDS-PAGE and fluorography. Monoclonal antibody 16-48-2 recognised both native and SDS denatured radioactive UNC-53 products verifying that the protein translated in vitro was bona fide UNC-53. This result shows that immuno-precipitation is a useful tool in schemes to purify native protein and to identify UNC-53 protein complexes in biochemical experiments.

Example 3 - Actin sedimentation assays (8A variant).

Besides the N-terminal region of the protein which is similar to actin binding proteins, the predicted protein sequence of UNC-53 identified two putative actin binding sites. The first borders on the 3' end of the region of α-actinin/β-spectrin homology and the second lies in the 3' end of the cDNA sequence. This suggests that UNC-53 could potentially

bind two actin molecules and via actin cross-linking, stabilise a particular growth cone spike to promote directional extension. Alternatively, the two actin binding sites may serve to anchor UNC-53 (and its shorter gene products) to the microfilament cytoskeleton to then transduce a signal via the NTPase domain to the downstream pathway.

To test the two site model, full length and truncated versions of UNC-53 (pTB50 and pTB52) were transcribed and translated in rabbit reticulocyte lysates for 90 minutes following standard protocols (Promega). To remove insoluble components, the reactions were airfuged for 1 hour at 100,000 x g and the supernatant containing ^{35}S labelled UNC-53 products introduced in actin co-sedimentation assays according to the method of Vancompernolle et al. (1992), EMBO J. 11, 4739-4746. In this procedure, radioactively labelled UNC-53 was incubated with monomeric G-actin in G buffer (2 mM Tris pH 7.5, 0.2 mM $CaCl_2$ 0.5 mM β-mercaptoethanol, 0.2 mM ATP) for one hour at room temperature. The salt concentration was then increased with F buffer (1 M KCl, 10 mM $MgCl_2$) to a final concentration of 100 mM to promote polymerisation of G-actin to F-actin. After an additional one hour incubation, polymerised F-actin/protein complexes were pelleted at 100,000 x g in an airfuge, washed with G buffer, resuspended in Laemmli buffer and separated by denaturing SDS-PAGE. The presence of actin in the pellets was confirmed by Coomasie staining while radioactively labelled UNC-53 products were detected by fluorography. Both the full length UNC-53 protein, pTB50, and the truncated construct, pTB52 translated in vitro in rabbit reticulocyte lysates cosedimented with F-actin at starting G-actin concentrations of 50-100 μg/ml. This suggests that UNC-53 binds to microfilament

cytoskeleton. Moreover, deletion of the first putative actin binding site (pTB52) did not eliminate actin binding.

Example 4 - UNC53 interacts with F-actin cytoskeleton (7A and 8A variant)

Analysis of the predicted protein sequence of UNC-53 identified two putative actin binding sites of the LKK class. The first borders the 3' end of the region of α-actinin/ß-spectrin homolgy in the amino terminus of the protein while the second lies in the 3' end of the protein sequence upstream of the putative nucleotide binding domain. A single UNC-53 monomer could thus potentially bind and crosslink two actin molecules.

To test whether UNC-53 associates with the actin cytoskeleton, a 7A (pTB72) and 8A version (pTB73) of unc-53 (Figures 25 and 27 respectively) were transcribed and translated in rabbit reticulocyte lysates and the ^{35}S labelled products introduced into F-actin co-sedimentation assays (Figure 35a). The full length UNC-53 protein (pTB72) translated *in vitro* cosedimented with F-actin at starting G-actin concentrations of 100 mg/ml (Figure 35b) suggesting that UNC-53 interacts with F-actin. By 250 mg/ml, all of the UNC53 protein co-sedimented with the F-actin pellet. In contrast, no UNC53 was present in the pellet of the control reaction without actin. Thus, sedimentation was purely actin dependent. This result also indicated that the *in vitro* UNC-53 protein remained soluble even after the salt concentration was raised.

Deletion of the first putative actin binding site

in pTB73 did not eliminate actin binding since the larger pTB73 products, including the largest fragment co-sedimented with F-actin under the identical set of conditions (Figure 35b). However, since the rabbit reticulocyte lysates contain numerous proteins, it is possible that the interaction of UNC-53 with actin may not be direct but rather mediated through another associated protein.

Several smaller radiolabelled protein fragments in the TnT reactions were observed in addition to the predicted protein products. Immunoprecipitation experiments confirmed that these products were UNC53 derived. Most likely they result from additional translational starts at internal methionines, since the identical set of smaller products was observed from reaction to reaction; or from premature termination and proteolytic degradation. Many of these smaller fragments also co-sedimented with F-actin. Since the second predicted actin binding site is within the 3' end of the molecule, truncated proteins that are the result of internal starts would be expected to have this site and to bind actin.

EXPERIMENTAL PROCEDURES:

Construction of UNC53 plasmids.

The complete unc53 cDNA was cloned as a 5.1 kb NotI-ApaI cassette in the mammalian expression vector pCDNA3 (Invitrogen) to generate plasmid pTB72, the 7A clone variant. To optimize translational initiation at the first methionine, a mammalian KOZAK consensus sequence was engineered upstream of the start methionine by PCR amplification of DNA coding for the first 139 amino acids of the amino terminus with the

oligonucleotides BG03 (5'-ataagaatgcggccgccgccatgacgacgtcaaatgtagaattgata-3') and BG02 (5'-cgcggatcctcaaaccgcgggtggcataatggatg-3'). BG03 contains the mammalian KOZAK consensus sequence in addition to a NotI restriction site. pTB73 is a deletion of the first 408 base pairs of the unc53 cDNA contained in the vector Bluescript II-KS. This construction removes the first two methionines of the unc53 cDNA sequence such that the first possible start methionine in pTB73 is at amino acid position 165 in the cDNA sequence. In all these constructs, (pTB72, pTB73 and pTB50) the unc53 cDNA is inserted into the multiple cloning site such that the T7 promoter is immediately upstream of the 5' end of the cDNA sequence.

The first 139 amino acids of the UNC53 cDNA were amplified by PCR with oligonucleotides BG01 (5'ggaattccaaccatatgacgacgtcaaatgtagaattgaata-3') and BG02 (5'-cgcggatcctcaaaccgcgggtggcataatggatg-3') to generate a convenient NdeI cloning site immediately upstream of the start methionine. This amplification was cloned as an NdeI-BamHI fragment into the prokaryotic expression vector pRK172 (Godedert M. and Jakes R. (1990), EMBO J. Vol. 9, pp 4225-4230 and McLeod M *et al*, 1987 EMBO. J. Vol 6, pp 729-736) to generate construct pTB57. pTB61 contains the PCR derived amino terminus of pTB57 in addition to the 3' end of pTB50. Thus pTB61 contains the identical unc53 8A variant cDNA as in pTB50, but as an NdeI-NcoI fragment in the vector pRK172 for prokaryotic expression.

In vitro transcription/ translation reactions

The UNC53 cDNA constructs pTB72, pTB73 or pTB50 were transcribed and translated for 90' at 30°C in a cell free T7 polymerase expression system in rabbit reticulocyte lysates following the company's protocols (ProMega). Prior to further manipulations, the reactions were centrifuged for 1 hour at 100,000 x g to remove insoluble components. In all subsequent experiments, the supernatant containing the soluble fraction of ^{35}S labelled UNC-53 products was utilized.

Actin co-sedimentation assays

Soluble radioactively labelled ^{35}S-Met-UNC53 products were introduced in actin co-sedimentation assays according to the method of Vancompernolle et al. (1992). In this procedure, radioactively labelled UNC-53 was incubated with monomeric G-actin in G buffer (2 mM Tris-pH 7.5, 0.2 mM CaCl2, 0.5 mM b-mercaptoethanol, 0.2 mM ATP) for one hour at room temperature and then the salt concentration increased with F buffer (1 M KCl, 10 mM MgCl2) to a final concentration of 100 mM to promote polymerization of G-actin to F-actin. After an additional one hour incubation, polymerized F-actin/protein complexes were pelleted at 100,000 x g in an airfuge (Beckman), washed with G buffer, resuspended in Laemmli buffer and separated by denaturing SDS-PAGE. The presence of actin in the pellets was confirmed by Coomasie staining while radioactively labelled UNC-53 products were detected by fluorography. Briefly, after destaining, gels were soaked in 45 % methanol, 7.5 % acetic acid (vol/vol) for 30 minutes, followed by 30 min. in dimethyl sulfoxide (DMSO), and 1 hour in 10 % PPO dissolved in DMSO (wt/vol). The scintillant was precipitated by rehydrating the gels with four five

minute water washes. After drying, gels were exposed to Xray film (Hyperfilm-Amersham).

Immunoprecipitations

To confirm that the radioactively labelled proteins translated *in vitro* were of UNC53 origin, an anti-rat monoclonal antibody, 16-48-2, elicited against a 47 kD fragment of the 3' end of UNC-53 was used to immunoprecipitate UNC-53 proteins. In this experiment, the unc-53 construct pTB50 was transcribed and translated *in vitro* in rabbit reticulocyte lysates. The resulting radioactively labelled ^{35}S UNC-53 gene products were incubated with the monoclonal antibody under both denaturing (0.4% SDS, 2.0% Triton X-100) and non-denaturing conditions for 1 hour at room temperature, then incubated with protein G sepharose for 2 hours at room temperature, the beads washed 3 times with PBS and the bound products analyzed by SDS-PAGE and fluorography. Monoclonal antibody 16-48-2 recognized both native and denatured radioactive UNC-53 products. As a control, a reaction without monoclonal antibody 16-48-2 was treated identically.

<u>Example 5</u> - Interaction of UNC-53 with SEM-5/GRB-2

The observation that certain alleles of UNC-53 enhance the sex myoblast migration defect of sem-5 mutants is difficult to interpret. While the genetics suggests that UNC-53 and SEM-5 cooperate to regulate sex myoblast migration, it is unclear whether this is the result of a direct molecular interaction. To answer this question, two types of biochemical experiments were used to determine if UNC-53

physically interacts with SEM-5. In the first experiment, radioactively labelled ^{35}S UNC-53, synthesised in reticulocyte lysates, was incubated with SEM-5/GST (glutathione-S-transferase) fusion protein bound to glutathione resin or with GST protein bound to glutathione resin. After incubation, the beads were washed and the bound proteins analysed by SDS-PAGE and fluorography. This demonstrated that UNC-53 made in vitro specifically bound to the SEM-5/GST fusion protein resin. The GST fusion proteins have been previously described. Purification of GST-fusion proteins was facilitated by using a commercially available kit (Pharmacia). All purification methods followed the manufacturer's protocols.

To further characterise the nature of the interaction with SEM-5, a second experiment utilised Western blot overlays. UNC-53 fusion proteins were expressed in E.coli and the denatured protein lysates separated by SDS-PAGE and blotted to Immobilon-P nylon membrane (Milipore). Blots were incubated with biotin labelled SEM-5/GST, GRB-2/GST or GST protein, washed and bound multi-protein biotinylated complexes detected by probing with an avidin-alkaline phosphatase conjugate. The results from this experiment demonstrated that SEM-5 and its mammalian homologue GRB2 can interact with UNC-53 in vitro. Binding was observed in induced cell lysates only and probing with the UNC-53 monoclonal antibody 16-48-2 detected the identical sets of products. In addition, only the full length UNC-53 fusion, pTB61 (Fig. 7), which contained the SH3 binding sites gave a positive result (pTB52 was not tested) No signal was detectable for either of the SH3 binding site minus fusion proteins, pTB57 (Fig. 11) or pTB65 (Fig. 11). This provides supportive evidence that the polyproline

repeats of the UNC-53 directly bind to the SH3 domains of SEM-5. Moreover, these results show that a SEM-5 or GRB-2/GST glutathione resin may be used in schemes to affinity purify native UNC-53 from tissue culture cells or nematodes or other organism extracts.

Detailed Methodology

Radioactively labelled ^{35}S UNC-53 synthesized in reticulocyte lysates was incubated with SEM-5/GST (glutathione-S-transferase) fusion protein bound to glutathione resin or with GST protein alone bound to glutathione resin for one hour at 20°C. After incubation, the beads were washed four times with Phosphate Buffered Saline (PBS)/Triton X-100 (0.2%) and the bound proteins analyzed by SDS-PAGE and fluorography. The SEM5 and GRB2 GST fusions have been previously described (Lowenstein et al., 1992; Stern et al., 1993). Purification of GST-fusion proteins was facilitated using a commercially available kit (Pharmacia). All purification methods followed the company protocols.

Western blot overlays

Approximately 500-1000 mg each of purified GRB2-GST protein or GST protein were biotin labelled by the following procedure. After overnight dialysis in PBS at 4°C, 1 M Hepes, pH7.4, was added to a final concentration of 100 mM and 50-100 mg of biotinylation reagent, dissolved in dimethyl sulfoxide, and the mixture incubated at 20°C for 90 minutes. The biotinylation reaction was stopped by the addition of 1 M Tris, pH7.4 to a final concentration of 100 mM and the labelled proteins stored on ice.

The UNC-53 construct pTB61 was expressed in *E. coli* strain BL21 (DE3), and the denatured protein

lysate separated by SDS-PAGE and electroblotted to Immobilon-P nylon membrane (Millipore). Membranes were blocked with 1 % skim milk powder in TBS-T (20 mM Tris, pH7.6; 0.14 M NaCl; 0.1% Tween-20) for 1 hour at 37°C. Subsequently, membranes were incubated in equimolar amounts of either biotin labelled GRB-2/GST or biotin labelled GST protein for 1 hour at 20°C, washed 4 x with TBS-T and bound multi-protein biotinylated complexes detected by probing for 1 hour at 20°C with an avidin-alkaline phosphatase conjugate (dilution 1:5000). Biotinylated protein conjugate complexes were visualized with a chromogenic solution containing bromochloroindolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) in 100 mM Tris(pH 9.5), 100 mM NaCl, 5 mM $MgCl_2$.Development was terminated with 10 mM Tris (pH8.0), 1 mM EDTA.

<u>Example 6</u> - Transgenic Analysis

To further our understanding of the function of unc-53 we developed an <u>in vivo</u> assay to test gene fusions generated <u>in vitro</u>. Nematode expression vectors containing the full length unc-53 cDNA, TB3M5, downstream of various tissue specific and inducible promoters were constructed.

The mec-7 promoter of pTB112 (Fig. 7) confers tissue specific expression to the mechanosensory neurons, the unc-54 promoter of pTB111 (Fig. 7) confers tissue specific expression to body wall muscle and the hsp16-41 promoter of pTB109 (Fig. 7) confers and pTB110 (Fig. 7) confers heat inducible expression to somatic cells. pTB109 is a transcriptional fusion containing only the hsp16-41 gene promoter and has been shown to confer high levels of inducible expression in embryos. pTB110 contains a larger

portion of the hsp16-41/2 intergenic region in addition to a synthetic intron. This plasmid is expected to be highly inducible in embryos and post-embryonic stages in most somatic cell types.

Oocytes of both wild type (N2) and unc-53(n152) hermaphrodites were microinjected according to the method of Fire (1986), EMBO J., 5, 2673-2680. Coinjection of the unc-53 fusion with a selection plasmid, pRF4, a dominant marker of rol-6, allowed identification of transgenic animals by their right rolling phenotype (Mello et al, (1991), EMBO J., 10, 3959-3970. In C. elegans, the injected DNA does not integrate into the genome but rather forms extrachromosomal arrays which are heritable at a frequency ranging from 20-95% (Stinchcomb et al, (1985), Mol. Cell. Biol., 5, 3483-3496; Fire et al, (1990), Gene, 93, 189-198; Mello et al, (1991), EMBO J., 10, 3959-3970. Transgenic extrachromosomal lines were considered stable after the rolling phenotype had passed through four generations. Some transgenic HS-unc-53 strains were mutagenised with 3550 rads of γ rays emanating from a ^{60}Co source which produces breaks in the chromosomes allowing for insertion of the extrachromosomal array. Stable integrants were identified by screening for homozygous rolling F3 broods. The names and genotypes of all transgenic strains are listed in Table 1 with details of the unc-53 fusions (constructs/vectors) listed in Table 2:

Table 1 - Extend in other constructs

STRAIN NAME	PARENTAL STRAIN	unc53 FUSION	SELECTION	lacZ MARKER
TB3In54	n152	pTB109	pRF4	UL6
TBAIn8	N2	pTB110	pRF4	pPCZ1

TBAIn61	N2	pTB110	pRF4	pPCZ1
TBAIn69	N2	pTB110	pRF4	pPCZ1
TBAIn76 Accession No 1385CB (See Fig 17A)	N2	pTB110	pRF4	pPCZ1
TBAIn90	N2	pTB110	pRF4	pPCZ1
TBAIn210	N2	pTB110	pRF4	pPCZ1
TBAIn222	N2	pTB110	pRF4	pPCZ1
TBAIn306	N2	pTB110	pRF4	pPCZ1
TBAIn327	N2	pTB110	pRF4	pPCZ1
TBBIn3	N2	pTB110	pRF4	pPCZ1
TBBIn267	N2	pTB110	pRF4	pPCZ1
TB1Ex10	n152	pTB112	pRF4	none
TB1Ex23	n152	pTB112	pRF4	none
TB1Ex8	N2	pTB112	pRF4	none
TB1Ex16	N2	pTB112	pRF4	none
TB2Ex1	N2	pTB112	pRF4	none
TB2Ex37	N2	pTB112	pRF4	none
TB3Ex10	N2	pTB112	pRF4	none
TB3Ex12	N2	pTB112	pRF4	none
TB3Ex20	N2	pTB112	pRF4	none
TB3Ex37	N2	pTB112	pRF4	none
TB4Ex14	N2	pTB112	pRF4	none
TB4Ex18	N2	pTB112	pRF4	none
TB4Ex22	N2	pTB112	pRF4	none
TB4Ex25 Accession No LMBP 1384CB (See Fig 16)	N2	pTB112	pRF4	none
TB1Ex3	n152	pTB111	pRF4	none

TB1Ex6 (See Fig 17B, C)	n152	pTB111	pRF4	none
TB1Ex11	n152	pTB111	pRF4	none

Notes for Table 1:
Ex-extrachromosomal
In-integrated
pTB109, pTB110-Heat shock unc-53 fusions
pTB111-mec-7 fusion
pTB112-unc-54 fusion
pRF4-rol-6 (su1006) (Mello et al, (1991), EMBO J., 5, 3959-3970)
UL6-excretory canal promoter lacZ fusion
pPCZ1-Hsp16-48/1 lacZ fusion (Stringham et al, (1992) Molec.Biol.Cell 3, 221-233)

Table 2

Full length cDNA tb3M5 (still has SL1 and 5' UTR)

pTB50 (NotI-ApaI fragment in Bluescript II-KS, for *in vitro* transcription)

pTB51 (NotI-ApaI fragment in Bluescript II-SK, for *in vitro* transcription)

pTB54 (NotI-ApaI fragment in pCDNA3, for mammalian expression)
(Deposited as accession no. LMBP3296)

pTB109 (NotI-ApaI fragment in hsp16-pucBM21, for *in vivo* expression)

pTB67 (NotI-Apa fragment in pGEM5 +)

PCR1 of amino terminus of cDNA
(*PCR using oligos BG01 and BG02)

pTB57 (NdeI-BamHI fragment in pRK172, for *E. coli* expression)

pTB58 (NdeI-NcoI fragment in pGEM5)

pTB63 (SacI-NcoI fragment in pRSETA, for E. coli expression)

pTB64 (BamHI fragment in pBluescriptII-KS)

Full length cDNA utilizing PCR1 amino terminus

pTB61 (NdeI-NcoI fragment in pRK172, for E. coli expression)

pTB110 (XbaI-KpnI fragment in pPD49.83, for in vivo expression)

pTB111 (XbaI-KpnI fragment in pPD52.102, for in vivo expression)

pTB112 (XbaI-KpnI fragment in pPD30.38, for in vivo expression)
(Deposited as accession no. LMBP3295)

PCR2 of amino terminus of cDNA
(*PCR using oligos BG03 and BG01)

pTB59 (NotI-BamHI fragment in pBluescript II-KS)

pTB60 (NotI-XhoI fragment in pCDNA3, for mammalian expression)

Full length cDNA utilizing PCR2 amino terminus

pTB55 (NotI-EaeI fragment in pBluescriptII-KS)

pTB56 (NotI-ApaI fragment in pCDNA3, for mammalian expression)

Other constructs

pTB52 (SacII deletion of amino terminus of pTB50)

pTB53 (SacII deletion of amino terminus of pTB51)

pTB62 (SmaI fragment of pTB52 in pGEX2T, for prokaryotic expression)

pTB65 (NdeI-NcoI fragment of 3′ terminus in pRK172, for prokaryotic expression)

pTB66 (NdeI-EcoRI fragment of 3′ terminus in pRK172, for prokaryotic expression, MAB 16-48-2)

Initially, the phenotype of each transgenic line was characterised by inspection with a dissecting microscope and/or Nomarski optics. Transgenic strains were directly analysed for expression of unc-53 by immunohistochemistry. Briefly, embryos were adhered to polylysine coated slides and permeabilised by a combination of freeze fracturing and immersion in cold methanol and acetone (3-4 minutes each). Embryos were rehydrated through an acetone/distilled water series and then incubated for 30 minutes at room temperature in TBS-Tween (0.1%). The anti-UNC-53 monoclonal 16-48-2 anti-sera was applied undiluted and the slides incubated at 4°C overnight. The embryos were washed three times with TBS-T and then incubated in a secondary rhodamine like (Cy3-M)conjugated antibody for 1 hour at 37°C. After 3-4 washed in TBS-T the slides were mounted for fluorescence microscopy in 2% propylgallate, 80% glycerol-pH 8.0.

Characterisation of transgenic strains carrying pTB112

UNC-53 was over-expressed in the muscle of wild type animals (pTB112 in N2). Each extrachromosomal pTB112/N2 line consisted of wild type and rolling animals as expected, but in addition, several mutant phenotypes were observed at low frequency. These animals varied considerably in phenotype and included embryos which arrested at the two fold stage, larvae which hatched but died soon afterward, animals with extra protrusions on the epidermis and animals with a truncated posterior end. This phenotype is consistent with that of the mup or mua classes of muscle mutants in which the positioning and/or integrity of muscle attachments to the hypodermis has been disrupted. Most of these animals were either inviable or sterile. The progeny of the viable mutants contained the same

frequency of rollers, wild type and mutants as did the progeny of rolling individuals. Since the extrachromosomal array may be lost at each cell division, every animal is a mosaic. The healthy rollers may have lost the transgene from most muscle cells and may represent weak phenotypes whereas the 2 fold arrests represent the situation where the array has been lost from few muscle cells. Nomarski and polarised light microscopy of the severe larval lethals showed that the muscle cells were disorganised and over-extended.

Detailed analysis of the underlying defect in embryonic development that leads to this terminal phenotype were performed with immunofluorescence microscopy (Fig 21).

Since the unc-54 gene encodes the myosin heavy chain, we expected that this promoter would be active in body muscle descendants from the comma stage onwards. In the unc-54 - unc-53 strains, signal was indeed localised to the body muscle cells in comma and later stages as predicted. The immunofluorescence was localised to the cytoplasm of the cell bodies and was particularly intense at the tips of the extending processes. Increased process length was observed very early in muscle development (comma to 1.5 fold stage) and increased up to the three fold stage. No other abnormalities in shape or muscle myofilament pattern were observed in the anterior-posterior axis of the animal. Two and three fold embryos which were stained with the monoclonal antibody NE8(4c6.3) (Goh and Bogaert,(1991), Dev. Biol. 56, 110-156) appeared to have a relatively wild type myofilament structure. As these animals are mosaic, it may be possible that severe cases die in late morphogenesis and those which survive through embryogenesis to adulthood can tolerate a few distorted muscle cells.

pTB111 transgenic lines

Immunostains indicates that the transgene is expressed efficiently in the mechanosensory neurons of a transgenic extrachromosomal line carrying the pTB111 transgene in an unc-53 (n152) genetic background (Fig 20).

pTB109 and pTB110 lines

Twelve integrated lines derived from three separate mutageneses of extrachromosomal lines have been isolated. TB3In54 carries the pTB109 fusion in addition to pRF4. Nine TBA strains were isolated after mutagenesis of an extrachromosomal strain, HSA. There are two strains (TBB) derived from mutagenesis of the extrachromosomal strain HS B. Both TBA and TBB strains contain the transgenes pTB110, pPCZ1 and pRF4. Inclusion of the HS-lacZ plasmid, pPCZ1 (Stringham et al, (1992), Molec.Bio.Cell 3, 221-233) allows one to monitor the strength of the heat shock induction by assaying for β-galactosidase activity.

Immunostains of embryos freeze fractured after a two hour heat shock showed that the signal was most prominent in the pharynx, gut and neurons. Surprisingly, the signal had a speckled appearance. This may be a feature of heat shock. Heat shock proteins may sequester UNC-53 to "chaperone" it during stress. Alternatively, UNC-53 may be targeted for degradation. In one experiment, embryos were heat shocked for two hours, allowed to recover overnight and then freeze fractured the next morning. While levels were reduced, there was a little residual UNC-53 signal in the gut cells. Thus, about 16 hours later most the protein has gone.

Level of heat shock and recovery times are

therefore important factors in the mutant rescue experiments and the preferred assay system described in example 10. In addition, experiments suggest that heat shock induction in liquid culture versus agar plates or dry incubators versus water baths need careful calibration.

After a strong three hour heat shock, a high percentage of animals were not able to recover from the stress. Embryos which were not subjected to a double shock (2-two hour heat shocks at 33°C separated by a two-hour recovery) hatch out as malformed worms reminiscent of the muscle overexpression lines (Fig 21). The heat shock promoter used is especially active in the pharynx. Consistent with this, a strong pharyngeal morphogenetic phenotype was observed (Fig 21). Pharyngeal phenotypes are easy to score and quantify (feeding rate, dye uptake, LacZ lines staining the pharynx) by anyone skilled in the C. elegans field and may form a preferred embodiment of the assay.

Example 7

Over-expression of UNC-53 results in directional over-extension : Assay with 7A variant.

In wild type *C. elegans*, body muscle cells are normally spindle shaped while in UNC53 mutants, a number of these cells have a reduced process which results in a fork shaped tip. This phenotype is consistent with the general reduction of extension observed in many growth cone types along the longitudinal axis of the animal in unc-53 mutants. Recalling the extremely limited pattern of UNC53 expression in embryogenesis detected by immunostaining with monoclonal antibody 16-48-2;no UNC53 activity was

discernable in wild type body muscle cells during outgrowth suggesting that the levels of UNC53 activity required for this extension may be extremely low.

We overexpressed unc-53 in the muscle of wild type animals by expressing the full length cDNA under the control of the unc-54 myosin heavy chain promoter in the fusion pTB113. Plasmid pTB113 is a translational fusion containing the 7A variant unc-53 cDNA sequence as an XbaI-KpnI fragment starting from the first methionine and including the unc-53 cDNA poly adenylation tail under control of the myosin heavy chain unc-54 promoter of the nematode expression vector pPD30.38 available on Internet web site ftp archive: ciwl, ciwemb.edu. Plasmid pTB114 contains the identical cDNA fragment under control of the hsp16-41 *-2* promoter (Jones et al.,1995, Dev. Biol. VOL. 171, PAGES 60-72) which confers heat inducible expression to somatic cells, in the expression vector pPD 49.83 (Fire, pers. comm.) The amino terminus of the UNC53 cDNA is identical to the PCR amplification with BG01 and BG02 of pTB57. Thus, both pTB113 and pTB114 are in frame translational fusions devoid of the SL1 leader sequence and upstream untranslated region of the cDNA.

Each transgenic mosaic line (3 were examined) consisted of wild type and rolling animals as expected, but in addition, several mutant phenotypes were observed at a low frequency. These animals varied considerably in phenotype and included, embryos which arrested at the two fold stage, larvae which hatched but died soon afterwards, animals with extra protrusions on the epidermis and animals with a truncated posterior end. Most of these latter animals

were either inviable or sterile. The progeny of the viable mutants contained the same frequency of rollers, wild type and mutants as did the progeny of rolling individuals. Since the extrachromosomal array may be lost at each cell division, every animal is a mosaic. The healthy rollers may have lost the transgene from most muscle cells and may represent weak phenotypes whereas the 2 fold arrests represent the situation where the array has been retained in most muscle cells. The truncated posterior end may be the result of lethality in the D lineage due to mosaicism. Nomarski and polarized light microscopy of the severe larval lethals showed that the muscle cells were disorganized and over-extended in the longitudinal axis. In some cases the muscle cells appeared detached from the hypodermis. As these animals are mosaic, it may be possible that severe cases die early in morphogenesis whereas those which survive through embryogenesis to adulthood can tolerate a few distorted muscle cells.

In transgenic pTB113 strains, UNC53 expression, as detected by immunostaining with monoclonal antibody 16-48-2, was localized to the body muscle cells in comma and later stages as predicted for the UNC-53 promoter (myosin heavy chain). The pattern of immunofluoresence with the anti UNC-53 antibody was localized to the cytoplasm of the cell bodies and was particularly intense at the tips of the extending processes and in the cytoskeleton, when compared to phalloidin staining which specifically stains the actin cytoskeleton. The identical pattern of sub-cellular localization, in the cytoplasm and cytoskeleton, was also observed in the intestinal

cells of pTB114 transgenic embryos expressing UNC-53 ectopically after heat shock.

In addition, the growth cone processes appeared to be overextended specifically in the anterior-posterior axis of the animal. To verify this, the length of body muscle cells over-expressing the UNC53 cDNA in the pTB113 strains were measured and compared to the length of wild-type muscle growth cones expressing an unc-54 promoter-GFP (green fluorescent protein) fusion, pPD49.83 (available on Internet Web Ste Ftp archive: ciwl. ciwemb.edu. The GFP reporter allowed visualization of the entire cell body and boundaries of the muscle cells in wild-type animals. We estimated that the processes of the pTB113 expressing cells were roughly 1½ times the length of pPD49.83 expressing wild type cells.

The lethality in the transgenic progeny of the three pTB113 strains examined ranged from 32% to 78%. Thus a significant proportion of the transformed mosaic progeny did not survive morphogenesis. In contrast, no lethality was observed in the pPD93.48 (unc-54-GFP) control strains. The lethality observed in the pTB113 lines is likely the consequence of overextension of muscle cells during embryogenesis because (a) both pTB113 and pPD93.48 utilize the identical promoter and should be expressed in the same cells at the same point in development, and (b) rol-6 selection was used to identify transformants for both constructs.

Example 8

Transient and stable transfection of UNC-53 in N4 neuroblastoma cells.

pTB72 and a plasmid expressing LacZ under the CMV promoter were transfected transiently with the Ca-phosphate method in N4 neuroblastoma cells.

N4 cells and their stably transfected counterparts were grown in Minimum Essential Medium (MEM)-REGA 3 (GIBCO BRL) supplemented with 10% Foetal Calf Serum, 1% L-Glutamine, 2% Sodium Bicarbonate, 200 units/ml penicilline and 200 μg/ml Streptomycine, in a humidified atmosphere of 90% air and 10% CO_2 at 37ºC. Transfections were performed by the Lipofectamine method (GIBCO BRL). 18 to 24 hrs before transfection cells were seeded in complete growth medium at a density of 7×10^5 per well in a six well tissue culture plate, and incubated at 37º C in a CO_2 incubator. For each transfection the following solutions were prepared.:

SolA = 4 μg of DNA diluted in 200 ul of Optimem (GIBCO BRL)

SolB = 12 ul of Lipofectamine reagent diluted in 200 ul of Optimem (GIBCO BRL)

Solutions A and B were combined, gently mixed and incubated at room temperature for 30 minutes. For each transfection 0.6 ml of Optimem was added to the lipid-DNA complex to reach the final volume of 1 ml. This mixture was then added onto the cells (which had been previously rinsed once with 2 ml of Optimem). The cells were incubated in the transfection mixture for 5 hrs at 37C in a CO2 incubator. At the beginning of the sixth hour from transfection, 1 ml of complete growth medium supplemented with 20% of Foetal calf serum was added to the transfected cells. The cells were incubated for 18 hrs at 37C in a CO2 incubator. 24 hrs following the beginning of transfection the supernatans was replaced with fresh growth medium. 72hrs post transfection cell cultures from each well were harvested, diluted 1:24 and distributed over 24

well plates with the growth medium containing 500, 750 ug/ml or 1mg/ml of geneticin (G418, GIBCO BRL). After ~12 days from the start of selection, single clones were picked and allowed to grow in the absence of selection. Of 27 initial clones, 7 were lost while expanding the clones because of their slow growth rate and the apparent general toxicity of caused by the transfected construct. Clone 9 was selected for further analysis.

Functional assay for neurite extension in N4 neuroblastoma

Step (1): Quantitative determination of neuronal morphology, i.e. length of neurites and fraction of positive cells is performed fully automatically. As an example we studied the degree of morphological differentiation in the wild-type N4 cells to a stably transfected C9 clone.

Step (2): Quantitative neuronal morphology

Morphological changes of neurones were quantitated as described in GEERTS et al (1992 Restorative Neurology and Neuroscience 4: 21-32 and Katsuhito *et al* Neurodegeration, 2: 173-181). Briefly, at appropriate times, glutaraldehyde was applied to cell cultures. No washing steps were performed. This ensured that the morphology of the cells at that time point was frozen. The cells were observed in transmitted light mode on an Axiovert microscope, equipped with a Marzhauser scanning stage driven by an Indy workstation (Silicon graphics). Images were captured using a MC5 video camera (HCS). About 3000 cells were detected in 64 neatly aligned images, forming a 8x8 square matrix of images. The exact alignment of the images ensured that neurites

could be followed from one image field to the next. The analysis software automatically detected cell bodies and neurites and saved cell body size and length of each individual neurite on a file. Different parameters were subsequently calculated. The neurite length per cell was calculated on freely lying cells (not within a cluster). The fraction positive cells is the fraction of cells having at least one neurite with a length exceeding twice the cell body diameter. Figure 40 clearly shows that clone C9 increases both neurite length (free length) and fraction of positive cells, compared to wild-type N4 cells clone.

Example 9

Transient and stable transfection of UNC-53 in MCF-7 breast carcinoma cells.

pTB72 and a plasmid expressing Lac Z under the CMV promoter where transfected transiently with the Ca-phosphate method in MCF-7 breast carcinoma cells.

MCF7 cells and their stably transfected counterparts were grown in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO BRL) supplemented with 10% foetal Calf Serum, 1% L-Glutamine, 1% of a 5mg/ml stock of Gentamicine and 1% of a 100mM stock of Sodium Pyruvate in an humidified atmosphere of 90% air and 10% CO2 at 37 C. Construct pTB72 was transfected by the Calcium-phosphate method (ref): 18-24hrs before transfection. cells were seeded at a density of $3x10^5$ in a six well tissue culture plate with complete growth medium. Two hours before transfection the culture medium was removed and replaced with 1.8 ml of fresh medium. The cells were put back in the incubator until the moment of transfection. DNA-$Ca_3(PO4)_2$ precipitates were prepared one hour before transfection : For each transfection (1 well): 4 ug of DNA (=3-4 ul) was

combined with 76 ul of TE (Tris HCl-EDTA pH 8) 0.1M to a final volume of 80 ul. To these DNA's diluted in TE, 20 ul of $CaCl_2$ Hepes solution was added to a final volume of 100 ul of DNA/$CaCl_2$ mixture. The 100 ul of DNA/$CaCl_2$ mixture was added very slowly, drop-by-drop to 100ul of 2x BS/Hepes while shaking, to a final volume of 200 ul. The resulting 200 ul DNA/Calcium Phosphate mixture was added to the cells and the mixture incubated for 8 hrs at 37 C in a CO_2 incubator. At the beginning of the ninth hour from the start of transfection, the supernatans with the DNA/Calcium phosphate mixture was replaced with 3 ml of complete culture medium. 72hrs post transfection, cells from each well were harvested, split1:24 in complete growth medium supplemented with 1mg/ml of Geneticin (G418, GIBCO-BRL) and plated out in 24 well plates. 15 days from the start of selection, single clones where picked and allowed to grow without selection. Three clones MCF7-pTB72-clone9, MCF7-pTB72-14 and MCF7-pTB72-15 were retained all of which have a similar phenotype.

1) Phenotyping UNC-53 transfected MCF-7 breast carcinoma cells:

The general morphology and motile behaviour of the three transfected MCF-7 clones are different from non-transfected cells.

The assay consists of a tyramide amplification of a classical immunofluorescent reaction. The cells were grown in defined medium with 10% charcoal treated serum and supplemented by 10 μg/ml insulin (final concentration) and 5 ng/ml basic fibroblast growth factor (final concentration). The substrate consisted of 50 μg/ml poly-L-lysine in chamber slides; cultures were maintained in a humidified atmosphere of 95/5% air/CO_2.

Inductin of expression of vimentin and of increased levels of fosfotyrosine was found in the transfected subclones. Vimentin formed dense clusters around the cell nucleus with some filamentous structures in the pseudo-podes. Fosfotyrosine, on the other hand, was predominantly found at the border of the cell ruffles, at the same subcellular area where UNC53 expression was found. This provides evidence of a controlling molecule functioning in a signal transduction pathway and that vimentin is an indicator of metastasis in cancerous cell lines.

2) Functional assay to establish the signal transduction role of UNC-53.

Cells locomote in tissues and on substrates. The type and amount of cell locomotion depends on different factors: (1) the physiological conditions perceived through receptors, which can be - for example - stimulation with or deprivation of serum, growth factor(s), cytokine(s), chemokine(s) or (pro-) inflammatory mediators; (2) the type and functionality of cell adhesion molecules expressed by cells and extracellular matrix molecules present in tissue or in culture model, (3) the actin, tubulin and/or intermediate filament cytoskeleton and (4) proper functioning of integrator proteins such as UNC-53, homologues or other molecules that translate physiological stimuli (or lack of stimuli) into increased or decreased cell motility, directional or random motility or different types of motility. Cell locomotion can be measured in different types of assays, such as disperse cells or in monolayer cultures, as cellular outgrowth from tissues in culture or in organotype cultures. Motility of live cells can be quantified microscopically as in example 8 or by time-lapse video or cinematography or by

phagokinetic assays (Albrecht-Buehler, 1977, Cell, 11:395) amongst other methods.

Cell motility assays are interesting tools to study the functioning and pharmacology of UNC-53 and the unc-53 pathway.

All previous observations were performed on MCF-7 cells grown in defined medium supplemented by 10 µg/ml insulin (final concentration) and 5ng/ml basic fibroblast growth factor (final concentration). This approach offers the possibility of investigating the role of FGF in the UNC53 role of signal transmission. Indeed, by comparing wild-type versus UNC53 transfected cells cultured in medium with or without FGF/insulin and/or by microinjection of UNC53 protein, it can be investigated if UNC53 is responsible directly for regulating a signal transduction pathway linking extracellular growth factors to the assembly of, amongst others, focal adhesions.

<u>Example 10</u>: Enhanced phagokinesis in Ce-unc-53 transfected MCF-7 cells.

In this example evidence is presented that transfection of a plasmid containing the Ce-unc-53 sequence under a suitable promoter enhances cell motility in the phagokinesis assay.

When culture plastics are coated with colloidal gold particles, a variety of cells types were shown to migrate over the plate and displace or phagocytose the gold lawn on their way while locomoting. The track left bare is a qualitative and quantitative measure of cell motility and/or locomotion. The basic methods have been described in detail elsewhere (Albrecht-Buehler, 1977, Cell, 11:395; Zetter, 1980, Nature, 285:41; O'Keefe et al., 1983, J. Invest. Dermatol., 85:130).

Methods

12 well plates were coated for 15 minutes with 5 μg/ml gelatin in water and gold coated as described by Albrecht-Bueller (1977). Ce-unc-53 transfected MCF-7 cells and the parent MCF-7 were cultured in parallel, trypsinised dispersed in culture medium and seeded in 12-well plates at a density of 2550 cells per well. The cells were allowed to adhere to the plate and to locomote for 16 hours. After incubation the cells were chemically fixed to the plate using paraformaldehyde, washed with distilled water and finally air-dried.

Subsequently, images of the gold lawns were captured using automated videomicroscopy, composite images of the wells were generated and single-cell phagokinetic tracks were measured using a home-made routine in SCIL™ software.

Results

The parent MCF-7 line displayed two cell populations with different motile behaviour in phagokinesis assays. In table 3 the fraction of parent and Ce-unc-53 transfected MCF-7 cells that produced linear tracks in the phagokinesis assay are shown. In the parent MCF-7 cells, 88% of the cells produce a round track (long and short axis less than 2-fold different) and 12% cells produce 'linear' tracks (long and short axis more than 2-fold different). Ce-unc-53 transfection of MCF-7 cells produced an increase of the fraction of cells displaying 'linear' tracks to 28% at the cost of the cells producing round tracks.

These observation suggest that Ce-unc-53 transfection into MCF-7 is capable of increasing *in situ* locomotion of MCF-7 e.g. by increased spreading, ruffling or other forms of non-directional motility in

FIG. 1.

```
                                   TB6 & TB3
                                   |                                    BSP1286
                                   |                                    HGIAI
GGTTTAATTACCCAAGTTTGAGACATCAATTCCATCGAACGAAATGTTGGTGCTCCGA AT
        10        20        30        40        50        60
                                        OUT OF FRAME ATG
        TTHIIII
        .AHAII
        ..  AATII                                            BANI
AAAATGACGACGTCAAATGTAGAATTGATACCAATCTACACGGATTGGGCCAATCGGC AC
        70        80        90        100       110       120
   M  T  T  S  N  V  E  L  I  P  I  Y  T  D  W  A  N  R  H
  ATG1
   ASUII            BBVI                               NRUI
CTTTCGAAGGGCAGCTTATCAAAGTCGATTAGGGATATTTCCAATGATTTTCGCGACT AT
        130       140       150       160       170       180
 L  S  K  G  S  L  S  K  S  I  R  D  I  S  N  D  F  R  D  Y

                                TB1B
                                |        ECORI          BSMI
CGACTGGTTTCTCAGCTTATTAATGTGATCGTTCCGATCAACGAATTCTCGCCTGCAT TC
        190       200       210       220       230       240
 R  L  V  S  Q  L  I  N  V  I  V  P  I  N  E  F  S  P  A  F

                              TB16
                              |BSTNI          AFLIII
                              |.              FOKI
ACGAAACGTTTGGCAAAAATCACATCGAACCTGGATGGCCTCGAAACGTGTCTCGACT AC
        250       260       270       280       290       300
 T  K  R  L  A  K  I  T  S  N  L  D  G  L  E  T  C  L  D  Y

                                          TB1
                    HPHI                  |ECORV    NSPBII
CTGAAAAATCTGGGTCTCGACTGCTCGAAACTCACCAAAACCGATATCGACAGCGGAA AC
        310       320       330       340       350       360
 L  K  N  L  G  L  D  C  S  K  L  T  K  T  D  I  D  S  G  N

   BBVI        MBOII
   .           .  NSPBII
   .           .  PVUII                             HINDIII
TTGGGTGCAGTTCTCCAGCTGCTCTTCCTGCTCTCCACCTACAAGCAGAAGCTTCGGC AA
        370       380       390       400       410       420
 L  G  A  V  L  Q  L  L  F  L  L  S  T  Y  K  Q  K  L  R  Q

                    FOKI
                    .  MBOII                              NSPBII
                    .  .                                  .SACII
CTGAAAAAAGATCAGAAGAAATTGGAGCAACTACCCACATCCATTATGCCACCCGCGG TT
        430       440       450       460       470       480
 L  K  K  D  Q  K  K  L  E  Q  L  P  T  S  I  M  P  P  A  V
                                           ATG 2
              AFLIII
TCTAAATTACCCTCGCCACGTGTCGCCACGTCAGCAACCGCTTCAGCAACTAACCCAA AT
        490       500       510       520       530       540
 S  K  L  P  S  P  R  V  A  T  S  A  T  A  S  A  T  N  P  N

        FOKI        HINCII BSTNI
TCCAACTTTCCACAAATGTCAACATCCAGGCTTCAGACTCCACAGTCAAGAATATCGA AA
        550       560       570       580       590       600
 S  N  F  P  Q  M  S  T  S  R  L  Q  T  P  Q  S  R  I  S  K
               ATG3
```

FIG. 1 CONTINUED. 2/99

```
     TB6B                                          AHAII
     |                                             .  AATII
ATTGATTCATCAAAGATTGGTATCAAGCCAAAGACGTCTGGACTTAAACCACCCTCAT CA
        610        620        630        640        650        660
 I  D  S  S  K  I  G  I  K  P  K  T  S  G  L  K  P  P  S  S

TCAACCACTTCATCAAATAATACAAATTCATTCCGTCCGTCGAGCCGTTCGAGTGGCA AT
        670        680        690        700        710        720
 S  T  T  S  S  N  N  T  N  S  F  R  P  S  S  R  S  S  G  N

                     ECORV                    MBOII
AATAATGTTGGCTCGACGATATCCACATCTGCGAAGAGCTTAGAATCATCATCAACGT AC
        730        740        750        760        770        780
 N  N  V  G  S  T  I  S  T  S  A  K  S  L  E  S  S  S  T  Y

          ASUII                                      XBAI
AGCTCTATTTCGAATCTAAACCGACCTACCTCCCAACTCCAAAAACCTTCTAGACCAC AA
        790        800        810        820        830        840
 S  S  I  S  N  L  N  R  P  T  S  Q  L  Q  K  P  S  R  P  Q

                                                   NHEI
ACCCAGCTAGTTCGTGTTGCTACAACTACAAAAATCGGAAGCTCAAAGCTAGCCGCTC CG
        850        860        870        880        890        900
 T  Q  L  V  R  V  A  T  T  T  K  I  G  S  S  K  L  A  A  P

             BSP1286
             HGIAI                            MBOII        BANII
             .                                .            BSP1286
AAAGCCGTGAGCACCCCAAAACTTGCTTCTGTGAAGACTATTGGAGCAAAACAAGAGC CC
        910        920        930        940        950        960
 K  A  V  S  T  P  K  L  A  S  V  K  T  I  G  A  K  Q  E  P

       NSPBII                       BSMI                      MBOII
GATAACAGCGGTGGTGGTGGTGGTGGAATGCTGAAATTAAAGTTATTCAGTAGCAAAA AC
        970        980        990        1000       1010       1020
 D  N  S  G  G  G  G  G  G  M  L  K  L  K  L  F  S  S  K  N
                            ATG4

                                                  BANI
CCATCTTCCTCATCGAATAGCCCACAACCTACGAGAAAGGCGGCGGCGGTGCCTCAAC AA
        1030       1040       1050       1060       1070       1080
 P  S  S  S  S  N  S  P  Q  P  T  R  K  A  A  A  V  P  Q  Q

    BBVI
CAAACTTTGTCGAAAATCGCTGCCCCAGTGAAAAGTGGCCTGAAGCCGCCGACCAGTA AG
        1090       1100       1110       1120       1130       1140
 Q  T  L  S  K  I  A  A  P  V  K  S  G  L  K  P  P  T  S  K

                                            TB22
BSTXI                          HINDIII      |
CTGGGAAGTGCCACGTCTATGTCGAAGCTTTGTACGCCAAAAGTTTCCTACCGTAAAA CG
        1150       1160       1170       1180       1190       1200
 L  G  S  A  T  S  M  S  K  L  C  T  P  K  V  S  Y  R  K  T

  AHAII   HGAI                                   SFANI
GACGCCCCAATCATATCTCAACAAGACTCGAAACGATGCTCAAAGAGCAGTGAAGAAG AG
        1210       1220       1230       1240       1250       1260
 D  A  P  I  I  S  Q  Q  D  S  K  R  C  S  K  S  S  E  E  E
```

FIG. 1 CONTINUED. 3/99

```
     MBOII
     .BSPMII
     .. MBOII
TCCGGATACGCTGGATTCAACAGCACGTCGCCAACGTCATCATCGACGGAAGGTTCCC TA
      1270      1280      1290      1300      1310      1320
 S  G  Y  A  G  F  N  S  T  S  P  T  S  S  S  T  E  G  S  L

      BSMI
      SPHI
      . MBOII
      . NSII
AGCATGCATTCCACATCTTCCAAGAGTTCAACGTCAGACGAAAAGTCTCCGTCATCAG AC
      1330      1340      1350      1360      1370      1380
 S  M  H  S  T  S  S  K  S  S  T  S  D  E  K  S  P  S  S  D
   ATG5

GATCTTACTCTTAACGCCTCCATCGTGACAGCTATCAGACAGCCGATAGCCGCAACAC CG
      1390      1400      1410      1420      1430      1440
 D  L  T  L  N  A  S  I  V  T  A  I  R  Q  P  I  A  A  T  P

              SSPI
GTTTCTCCAAATATTATCAACAAGCCTGTTGAGGAAAAACCAACACTGGCAGTGAAAG GA
      1450      1460      1470      1480      1490      1500
 V  S  P  N  I  I  N  K  P  V  E  E  K  P  T  L  A  V  K  G

                 BINI XHOII           NSPBII
                  .    .              PVUII
GTGAAAAGCACAGCGAAAAAAGATCCACCTCCAGCTGTTCCGCCACGTGACACCCAGC CA
      1510      1520      1530      1540      1550      1560
 V  K  S  T  A  K  K  D  P  P  P  A  V  P  P  R  D  T  Q  P

                                              HINCII        ECORV
ACAATCGGAGTTGTTAGTCCAATTATGGCACATAAGAAGTTGACAAATGACCCCGTGA TA
      1570      1580      1590      1600      1610      1620
 T  I  G  V  V  S  P  I  M  A  H  K  K  L  T  N  D  P  V  I

                                   SFANI
TCTGAAAAACCAGAACCTGAAAAGCTCCAATCAATGAGCATCGACACGACGGACGTTC  CA
      1630      1640      1650      1660      1670      1680
 S  E  K  P  E  P  E  K  L  Q  S  M  S  I  D  T  T  D  V  P

CCGCTTCCACCTCTAAAATCAGTTGTTCCACTTAAAATGACTTCAATCCGACAACCAC CA
      1690      1700      1710      1720      1730      1740
 P  L  P  P  L  K  S  V  V  P  L  K  M  T  S  I  R  Q  P  P

   MBOII
ACGTACGATGTTCTTCTAAAACAAGGAAAAATCACATCGCCTGTCAAGTCGTTTGGAT AT
      1750      1760      1770      1780      1790      1800
 T  Y  D  V  L  L  K  Q  G  K  I  T  S  P  V  K  S  F  G  Y

 HGAI                              HGAI
 .                                 . MBOII
GAGCAGTCGTCCGCGTCTGAAGACTCCATTGTGGCTCATGCGTCGGCTCAGGTGACTC CG
      1810      1820      1830      1840      1850      1860
 E  Q  S  S  A  S  E  D  S  I  V  A  H  A  S  A  Q  V  T  P

  HPHI                                                FOKI
CCGACAAAAACTTCTGGTAATCATTCGCTGGAGAGAAGGATGGGAAAGAATAAGACAT CA
      1870      1880      1890      1900      1910      1920
 P  T  K  T  S  G  N  H  S  L  E  R  R  M  G  K  N  K  T  S

      NSPBII         AHAII  HGAI
GAATCCAGCGGCTACACCTCTGACGCCGGTGTTGCGATGTGCGCCAAAATGAGGGAGA AG
```

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FIG. 1 CONTINUED. 4/99

```
             NSPBII                ABAII   BGAI
GAATCCAGCGGCTACACCTCTGACGCCGGTGTTGCGATGTGCGCCAAAATGAGGGAGAAG
          1930      1940      1950      1960      1970      1980
 E  S  S  G  Y  T  S  D  A  G  V  A  M  C  A  K  M  R  E  K

                                         BSP1286
                                         BGIAI                ASUII
CTGAAAGAATACGATGACATGACTCGTCGAGCACAGAACGGCTATCCTGACAACTTCGAA
          1990      2000      2010      2020      2030      2040
 L  K  E  Y  D  D  M  T  R  R  A  Q  N  G  Y  P  D  N  F  E

              MBOII                                 BANII
                                                    BSP1286
              .                                     BGIAI
              .                                     SACI
              .
GACAGTTCCTCCTTGTCGTCTGGAATATCCGATAACAACGAGCTCGACGACATATCCACG
          2050      2060      2070      2080      2090      2100
 D  S  S  S  L  S  S  G  I  S  D  N  N  E  L  D  D  I  S  T

                  BSPMII
                  .  ACCI                                 FOKI
GACGATTTGTCCGGAGTAGACATGGCAACAGTCGCCTCCAAACATAGCGACTATTCCCAC
          2110      2120      2130      2140      2150      2160
 D  D  L  S  G  V  D  M  A  T  V  A  S  K  H  S  D  Y  S  H

               MBOII
               .  MBOII                  AVAI           AVAII
TTTGTTCGCCATCCCACGTCTTCTTCCTCAAAGCCCCGAGTCCCCAGTCGGTCCTCCACA
          2170      2180      2190      2200      2210      2220
 F  V  R  H  P  T  S  S  S  S  K  P  R  V  P  S  R  S  S  T

                  AVAI
                  XHOI
TCAGTCGATTCTCGATCTCGAGCAGAACAGGAGAATGTGTACAAACTTCTGTCCCAGTGC
          2230      2240      2250      2260      2270      2280
 S  V  D  S  R  S  R  A  E  Q  E  N  V  Y  K  L  L  S  Q  C

            BBVI BGLI
            .     . BANI
            .     . .AHAII
            .     . .NARI
            .     . ..   HAEII
            .     . ..   . NSPBII                     BINI XHOII
            .     . ..  . .                            .   . FOKI
CGAACGAGCCAACGTGGCGCCGCTGCCACCTCAACCTTCGGACAACATTCGCTAAGATCC
          2290      2300      2310      2320      2330      2340
 R  T  S  Q  R  G  A  A  A  T  S  T  F  G  Q  H  S  L  R  S

AVAI
.NCII
..NCII
..SMAI                                 NSPBII
...                                    PVUII
CCGGGATACTCATCCTATTCTCCACACTTATCAGTGTCAGCTGATAAGGACACAATGTCT
          2350      2360      2370      2380      2390      2400
 P  G  Y  S  S  Y  S  P  H  L  S  V  S  A  D  K  D  T  M  S
```

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FIG. 1 CONTINUED.

```
                    SPEI
                    .   SALI
                    .   .ACCI
                    .   ..HINCII
                    .   ...MBOII
ATGCACTCACAGACTAGTCGACGACCTTCTTCACAAAAACCAAGCTATTCAGGCCAAT TT
      2410      2420      2430      2440      2450      2460
 M  H  S  Q  T  S  R  R  P  S  S  Q  K  P  S  Y  S  G  Q  F

                          FOKI                          BSP1286
                          .                             HGIAI
CATTCACTTGATCGTAAATGCCACCTTCAAGAGTTCACATCCACCGAGCACAGAATGG CG
      2470      2480      2490      2500      2510      2520
 H  S  L  D  R  K  C  H  L  Q  E  F  T  S  T  E  H  R  M  A

                AVAI
                .BANII
                .BSP1286 BANI                MBOII     BINI BAMHI
                ..       .                   .         .    XHOII
GCTCTCTTGAGCCCGAGACGGGTGCCGAACTCGATGTCGAAATATGATTCTTCAGGAT CC
      2530      2540      2550      2560      2570      2580
 A  L  L  S  P  R  R  V  P  N  S  M  S  K  Y  D  S  S  G  S

  BINI       AVAI
TACTCGGCGCGTTCCCGAGGTGGAAGCTCTACTGGTATCTATGGAGAGACGTTCCAAC TG
      2590      2600      2610      2620      2630      2640
 Y  S  A  R  S  R  G  G  S  S  T  G  I  Y  G  E  T  F  Q  L

                                                   BINI BAMHI
                                                   .    XHOII
CACAGACTATCCGATGAAAAATCCCCCGCACATTCTGCCAAAAGTGAGATGGGATCCC AA
      2650      2660      2670      2680      2690      2700
 H  R  L  S  D  E  K  S  P  A  H  S  A  K  S  E  M  G  S  Q

BINI      NHEI          NDEI
.         .             .  XHOII   BINI
CTATCACTGGCTAGCACGACAGCATATGGATCTCTCAATGAGAAGTACGAACATGCTA TT
      2710      2720      2730      2740      2750      2760
 L  S  L  A  S  T  T  A  Y  G  S  L  N  E  K  Y  E  H  A  I

                                            SALI
                                            .ACCI
                                            ..HINCII
CGGGACATGGCACGTGACTTGGAGTGTTACAAGAACACTGTCGACTCACTAACCAAGA AA
      2770      2780      2790      2800      2810      2820
 R  D  M  A  R  D  L  E  C  Y  K  N  T  V  D  S  L  T  K  K

                                            HINDIII
CAGGAGAACTATGGAGCATTGTTTGATCTTTTTGAGCAAAAGCTTAGAAAACTCACTC AA
      2830      2840      2850      2860      2870      2880
 Q  E  N  Y  G  A  L  F  D  L  F  E  Q  K  L  R  K  L  T  Q

   BINI
   .   CLAI                                MBOII
CACATTGATCGATCCAACTTGAAGCCTGAAGAGGCAATACGATTCAGGCAGGACATTG CT
      2890      2900      2910      2920      2930      2940
 H  I  D  R  S  N  L  K  P  E  E  A  I  R  F  R  Q  D  I  A
```

FIG. 1 CONTINUED.

```
               FOKI
               .  SFANI                                   HAEII
CATTTGAGGGATATTAGCAATCATCTTGCATCCAACTCAGCTCATGCTAACGAAGGCG CT
      2950      2960      2970      2980      2990      3000
 H  L  R  D  I  S  N  H  L  A  S  N  S  A  H  A  N  E  G  A

 MBOII       HPHI
 .           .  HINCII  FOKI
 .           .  .       .  SFANI             CLAI   CLAI
GGTGAGCTTCTTCGTCAACCATCTCTGGAATCAGTTGCATCCCATCGATCATCGATGT CA
      3010      3020      3030      3040      3050      3060
 G  E  L  L  R  Q  P  S  L  E  S  V  A  S  H  R  S  S  M  S

                   ECOB   BBVI         MBOII
                   .      .            .   BANII
                   .      .            .   BSP1286
                   .      .            .   HGIAI
                   .      .            .   SACI
TCGTCGTCGAAAAGCAGCAAGCAGGAGAAGATCAGCTTGAGCTCGTTTGGCAAGAACA AG
      3070      3080      3090      3100      3110      3120
 S  S  S  K  S  S  K  Q  E  K  I  S  L  S  S  F  G  K  N  K

   BINI BAMHI
   .    XHOII
   .    .   MBOII
   .    .   .   BINI    HPHI                       MBOII
   .    .   .   .       .                          .  MBOII
AAGAGCTGGATCCGCTCCTCACTCTCCAAGTTCACCAAGAAGAAGAACAAGAACTACG AC
      3130      3140      3150      3160      3170      3180
 K  S  W  I  R  S  S  L  S  K  F  T  K  K  K  N  K  N  Y  D

        NDEI             XHOII
        .                .BSPMII BINI
GAAGCACATATGCCATCAATTTCCGGATCTCAAGGAACTCTTGACAACATTGATGTGA TT
      3190      3200      3210      3220      3230      3240
 E  A  H  M  P  S  I  S  G  S  Q  G  T  L  D  N  I  D  V  I

                 BANII
                 BSP1286
                 HGIAI
                  SACI ECOK      APALI
                 .    .         .   BSP1286
                 .    .         .   HGIAI
GAGTTGAAGCAAGAGCTCAAAGAACGCGATAGTGCACTTTACGAAGTCCGCCTTGACA AT
      3250      3260      3270      3280      3290      3300
 E  L  K  Q  E  L  K  E  R  D  S  A  L  Y  E  V  R  L  D  N

           BINI
           .BSP1286
CTGGATCGTGCCCGCGAAGTTGATGTTCTGAGGGAGACAGTGAACAAGTTGAAAACCG AG
      3310      3320      3330      3340      3350      3360
 L  D  R  A  R  E  V  D  V  L  R  E  T  V  N  K  L  K  T  E

                        HPHI            AVAII     MBOII
AACAAGCAATTAAAGAAAGAAGTGGACAAACTCACCAACGGTCCAGCCACTCGTGCTT CT
      3370      3380      3390      3400      3410      3420
 N  K  Q  L  K  K  E  V  D  K  L  T  N  G  P  A  T  R  A  S
```

F/G. 1 CONTINUED.

```
                                                                    SFANI
TCCCGCGCCTCAATTCCAGTTATCTACGACGATGAGCATGTCTATGATGCAGCGTGTA GC
      3430      3440      3450      3460      3470      3480
 S  R  A  S  I  P  V  I  Y  D  D  E  H  V  Y  D  A  A  C  S

 BBVI      MBOII     ASUII
 .         .         .BINI
 .         .         .. BBVI
AGTACATCAGCTAGTCAATCTTCGAAACGATCCTCTGGCTGCAACTCAATCAAGGTTA CT
      3490      3500      3510      3520      3530      3540
 S  T  S  A  S  Q  S  S  K  R  S  S  G  C  N  S  I  K  V  T

                                   PVUI
                                   .   HINCII
                                   .   HPAI
                                   .   .   NCII
GTAAACGTGGACATCGCTGGAGAAATCAGTTCGATCGTTAACCCGGACAAAGAGATAA TC
      3550      3560      3570      3580      3590      3600
 V  N  V  D  I  A  G  E  I  S  S  I  V  N  P  D  K  E  I  I

      ECORV        HINCII
GTAGGATATCTTGCCATGTCAACCAGTCAGTCATGCTGGAAAGACATTGATGTTTCTA TT
      3610      3620      3630      3640      3650      3660
 V  G  Y  L  A  M  S  T  S  Q  S  C  W  K  D  I  D  V  S  I

                ACCI           SFANI                       CLAI
CTAGGACTATTTGAAGTCTACCTATCCAGAATTGATGTGGAGCATCAACTTGGAATCG AT
      3670      3680      3690      3700      3710      3720
 L  G  L  F  E  V  Y  L  S  R  I  D  V  E  H  Q  L  G  I  D

      SFANI   STYI                HGAI      AFLIII
      .       .                   .         MLUI
      .       .                   .         .HPHI   HGAI
GCTCGTGATTCTATCCTTGGCTATCAAATTGGTGAACTTCGACGCGTCATTGGAGACT CC
      3730      3740      3750      3760      3770      3780
 A  R  D  S  I  L  G  Y  Q  I  G  E  L  R  R  V  I  G  D  S

      FOKI
ACAACCATGATAACCAGCCATCCAACTGACATTCTTACTTCCTCAACTACAATCCGAA TG
      3790      3800      3810      3820      3830      3840
 T  T  M  I  T  S  H  P  T  D  I  L  T  S  S  T  T  I  R  M

         BANI                ACCI      AVAII  MBOII
TTCATGCACGGTGCCGCACAGAGTCGCGTAGACAGTCTGGTCCTTGATATGCTTCTTC CA
      3850      3860      3870      3880      3890      3900
 F  M  H  G  A  A  Q  S  R  V  D  S  L  V  L  D  M  L  L  P

                                                 AHAII
                                                 .  AATII
AAGCAAATGATTCTCCAACTCGTCAAGTCAATTTTGACAGAGAGACGTCTGGTGTTAG CT
      3910      3920      3930      3940      3950      3960
 K  Q  M  I  L  Q  L  V  K  S  I  L  T  E  R  R  L  V  L  A

                             BBVI      BSTNI
                             .         .    MBOII
GGAGCAACTGGAATTGGAAAGAGCAAACTGGCGAAGACCCTGGCTGCTTATGTATCTA TT
      3970      3980      3990      4000      4010      4020
 G  A  T  G  I  G  K  S  K  L  A  K  T  L  A  A  Y  V  S  I
```

SUBSTITUTE SHEET (RULE 26)

FIG. 1 CONTINUED. 8/99

```
ASUII                               MBOII      BSMI
CGAACAAATCAATCCGAAGATAGTATTGTTAATATCAGCATTCCTGAAAACAATAAAG AA
         4030       4040      4050      4060      4070      4080
 R   T   N   Q   S   E   D   S   I   V   N   I   S   I   P   E   N   N   K   E

     XMNI MBOII        AHAII
      .    .            .    BSTNI
      .    .            .    .    HGAI
      .    .            .    .    .   BGLII
      .    .            .    .    .   XHOII          SFANI        NSII
GAATTGCTTCAAGTGGAACGACGCCTGGAAAAGATCTTGAGAAGCAAAGAATCATGCA TC
         4090       4100      4110      4120      4130      4140
 E   L   L   Q   V   E   R   R   L   E   K   I   L   R   S   K   E   S   C   I

     XBAI
GTAATTCTAGATAATATCCCAAAGAATCGAATTGCATTTGTTGTATCCGTTTTTGCAA AT
         4150       4160      4170      4180      4190      4200
 V   I   L   D   N   I   P   K   N   R   I   A   F   V   V   S   V   F   A   N

                         AVAII                       HINCII ECORV
GTCCCACTTCAAAACAACGAAGGTCCATTTGTAGTATGCACAGTCAACCGATATCAAA TC
         4210       4220      4230      4240      4250      4260
 V   P   L   Q   N   N   E   G   P   F   V   V   C   T   V   N   R   Y   Q   I

      HPHI                                                FOKI
CCTGAGCTTCAAATTCACCACAATTTCAAAATGTCAGTAATGTCGAATCGTCTCGAAG GA
         4270       4280      4290      4300      4310      4320
 P   E   L   Q   I   H   H   N   F   K   M   S   V   M   S   N   R   L   E   G

                                                          FOKI
TTCATCCTACGTTACCTCCGACGACGGGCGGTAGAGGATGAGTATCGTCTAACTGTAC AG
         4330       4340      4350      4360      4370      4380
 F   I   L   R   Y   L   R   R   R   A   V   E   D   E   Y   R   L   T   V   Q

     MBOII
      .  SFANI
      .  .   BANII
      .  .   BSP1286
      .  .   HGIAI
      .  .   SACI        MBOII        MBOII
ATGCCATCAGAGCTCTTCAAAATCATTGACTTCTTCCCAATAGCTCTTCAGGCCGTCA AT
         4390       4400      4410      4420      4430      4440
 M   P   S   E   L   F   K   I   I   D   F   F   P   I   A   L   Q   A   V   N

                    ECORI                   AVAII        SPHI
AATTTTATTGAGAAAACGAATTCTGTTGATGTGACAGTTGGTCCAAGAGCATGCTTGA AC
         4450       4460      4470      4480      4490      4500
 N   F   I   E   K   T   N   S   V   D   V   T   V   G   P   R   A   C   L   N

                 BINI BAMHI
                  .   XHOII    BINI
TGTCCTCTAACTGTCGATGGATCCCGTGAATGGTTCATTCGATTGTGGAATGAGAACT TC
         4510       4520      4530      4540      4550      4560
 C   P   L   T   V   D   G   S   R   E   W   F   I   R   L   W   N   E   N   F

                 AFLIII                     BBVI
ATTCCATATTTGGAACGTGTTGCTAGAGATGGCAAAAAAAACCTTCGGTCGCTGCACT TC
         4570       4580      4590      4600      4610      4620
 I   P   Y   L   E   R   V   A   R   D   G   K   K   N   L   R   S   L   H   F
```

SUBSTITUTE SHEET (RULE 26)

FIG. 1 CONTINUED. 9/99

```
 BINI BAMHI
 .     XHOII     BINI  TTHIIII           EAEI                  NCII
CTTCGAGGATCCCACCGACATCGTCTCTAAAAAATGGCCGTGGTTCGATGGTGAAAAC CC
     4630      4640      4650      4660      4670      4680
 L  R  G  S  H  R  H  R  L

 HPHI    MBOII
 .       .BSP1286
 .       .HGIAI                     TTHIIII
 .       ..                         .HPHI    FOKI       BSPMI
GGAGAATGTGCTCAAACGTCTTCAACTCCAAGACCTCGTCCCGTCACCTGCCAACTCA TC
     4690      4700      4710      4720      4730      4740

                  AVAI
                  XHOI  BINI                SFANI
                  .     .                   . SPHI
CCGACAACACTTCAATCCCCTCGAGTCGTTGATCCAATTGCATGCTACCAAGCATCAG AC
     4750      4760      4770      4780      4790      4800

                         MBOII    MBOII                MBOII
CATCGACAACATTTGAACAGAAGACTCTAATCTTCTCTCGCCTCTCCCCCGCTTTCCT TA
     4810      4820      4830      4840      4850      4860

          BANI
          .   KPNI
TCTTCGTACCGGTACCTGATGATTCCCCATTTTCCCCCTTTTCCCCCCAATTTCCCAG AA
     4870      4880      4890      4900      4910      4920

                          AVAI
                          .NCII
                          ..NCII
                          ..SMAI
                          ... BANI   AHAII   HGAI   DRAI
CCTCCTGTTCCCTTTGTTCCTAGTCCTCCCGGGTGCCGACGCCGAAGCGATTTAAAAA CC
     4930      4940      4950      4960      4970      4980

                XMNI
TTTTTCTTTCCGAAACATTTCCCATTGCTCATTAATAGTCAAATTGAATAAACAGTGT AT
     4990      5000      5010      5020      5030      5040

GTACTTAAAAAAAAAAAAAAAAAAAAAAAAAAA
     5050      5060      5070
```

SUBSTITUTE SHEET (RULE 26)

10/99 FIG. 2.

COMPARISON OF 7A VS 8A CLONE

```
                                   TB6 & TB3
                                   |                               BSP1286
                                   |                               HGIAI
GGTTTAATTACCCAAGTTTGAGACATCAATTCCATCGAACGAAATGTTGGTGCTCCGAAT
         10        20        30        40        50        60

         TTHIIII
         .AHAII
         ..  AATII                                         BANI
AAAATGACGACGTCAAATGTAGAATTGATACCAATCTACACGGATTGGGCCAATCGGCAC
         70        80        90        100       110       120
    M  T  T  S  N  V  E  L  I  P  I  Y  T  D  W  A  N  R  H

    ASUII                 BBVI                         NRUI
CTTTCGAAGGGCAGCTTATCAAAGTCGATTAGGGATATTTCCAATGATTTTCGCGACTAT
         130       140       150       160       170       180
L  S  K  G  S  L  S  K  S  I  R  D  I  S  N  D  F  R  D  Y

                                   TB1B
                                   |           ECORI       BSMI
CGACTGGTTTCTCAGCTTATTAATGTGATCGTTCCGATCAACGAATTCTCGCCTGCATTC
         190       200       210       220       230       240
R  L  V  S  Q  L  I  N  V  I  V  P  I  N  E  F  S  P  A  F

                                 TB16
                                 |BSTNI          AFLIII
                                 |.              FOKI
ACGAAACGTTTGGCAAAAATCACATCGAACCTGGATGGCCTCGAAACGTGTCTCGACTAC
         250       260       270       280       290       300
T  K  R  L  A  K  I  T  S  N  L  D  G  L  E  T  C  L  D  Y

                                                 TB1
                        HPHI                     |ECORV   NSPBII
CTGAAAAAATCTGGGTCTCGACTGCTCGAAACTCACCAAAACCGATATCGACAGCGGAAAC
         310       320       330       340       350       360
L  K  N  L  G  L  D  C  S  K  L  T  K  T  D  I  D  S  G  N

    BBVI        MBOII
    .           . NSPBII
    .           . PVUII                           HINDIII
TTGGGTGCAGTTCTCCAGCTGCTCTTCCTGCTCTCCACCTACAAGCAGAAGCTTCGGCAA
         370       380       390       400       410       420
L  G  A  V  L  Q  L  L  F  L  L  S  T  Y  K  Q  K  L  R  Q

                        FOKI
                        .  MBOII                        NSPBII
                        .  .                            .SACII
CTGAAAAAAGATCAGAAGAAATTGGAGCAACTACCCACATCCATTATGCCACCCGCGGTT
         430       440       450       460       470       480
L  K  K  D  Q  K  K  L  E  Q  L  P  T  S  I  M  P  P  A  V

                  AFLIII
TCTAAATTACCCTCGCCACGTGTCGCCACGTCAGCAACCGCTTCAGCAACTAACCCAAAT
         490       500       510       520       530       540
S  K  L  P  S  P  R  V  A  T  S  A  T  A  S  A  T  N  P  N

        FOKI           HINCII BSTNI
TCCAACTTTCCACAAATGTCAACATCCAGGCTTCAGACTCCACAGTCAAGAATATCGAAA
         550       560       570       580       590       600
S  N  F  P  Q  M  S  T  S  R  L  Q  T  P  Q  S  R  I  S  K
```

F/G. 2 CONTINUED. 11/99

```
   TB6B                                          AHAII
    |                                           .  AATII
ATTGATTCATCAAAGATTGGTATCAAGCCAAAGACGTCTGGACTTAAACCACCCTCATCA
         610       620       630       640       650       660
 I  D  S  S  K  I  G  I  K  P  K  T  S  G  L  K  P  P  S  S

TCAACCACTTCATCAAATAATACAAATTCATTCCGTCCGTCGAGCCGTTCGAGTGGCAAT
         670       680       690       700       710       720
 S  T  T  S  S  N  N  T  N  S  F  R  P  S  S  R  S  S  G  N

                    ECORV                           MBOII
AATAATGTTGGCTCGACGATATCCACATCTGCGAAGAGCTTAGAATCATCATCAACGTAC
         730       740       750       760       770       780
 N  N  V  G  S  T  I  S  T  S  A  K  S  L  E  S  S  S  T  Y

          ASUII                                         XBAI
AGCTCTATTTCGAATCTAAACCGACCTACCTCCCAACTCCAAAAACCTTCTAGACCACAA
         790       800       810       820       830       840
 S  S  I  S  N  L  N  R  P  T  S  Q  L  Q  K  P  S  R  P  Q

                                                       NHEI
ACCCAGCTAGTTCGTGTTGCTACAACTACAAAAATCGGAAGCTCAAAGCTAGCCGCTCCG
         850       860       870       880       890       900
 T  Q  L  V  R  V  A  T  T  T  K  I  G  S  S  K  L  A  A  P

             BSP1286
             HGIAI                           MBOII         BANII
             .                               .             BSP1286
AAAGCCGTGAGCACCCCAAAACTTGCTTCTGTGAAGACTATTGGAGCAAAACAAGAGCCC
         910       920       930       940       950       960
 K  A  V  S  T  P  K  L  A  S  V  K  T  I  G  A  K  Q  E  P

        NSPBII                     BSMI                    MBOII
GATAACAGCGGTGGTGGTGGTGGTGGAATGCTGAAATTAAAGTTATTCAGTAGCAAAAAC
         970       980       990      1000      1010      1020
 D  N  S  G  G  G  G  G  G  M  L  K  L  K  L  F  S  S  K  N

                                                       BANI
CCATCTTCCTCATCGAATAGCCCACAACCTACGAGAAAGGCGGCGGCGGTGCCTCAACAA
        1030      1040      1050      1060      1070      1080
 P  S  S  S  S  N  S  P  Q  P  T  R  K  A  A  A  V  P  Q  Q

      BBVI
CAAACTTTGTCGAAAATCGCTGCCCCAGTGAAAAGTGGCCTGAAGCCGCCGACCAGTAAG
        1090      1100      1110      1120      1130      1140
 Q  T  L  S  K  I  A  A  P  V  K  S  G  L  K  P  P  T  S  K

                                          TB22
BSTXI                          HINDIII    |
CTGGGAAGTGCCACGTCTATGTCGAAGCTTTGTACGCCAAAAGTTTCCTACCGTAAAACG
        1150      1160      1170      1180      1190      1200
 L  G  S  A  T  S  M  S  K  L  C  T  P  K  V  S  Y  R  K  T

  AHAII   HGAI                                  SFANI
GACGCCCCAATCATATCTCAACAAGACTCGAAACGATGCTCAAAGAGCAGTGAAGAAGAG
        1210      1220      1230      1240      1250      1260
 D  A  P  I  I  S  Q  Q  D  S  K  R  C  S  K  S  S  E  E  E
```

F/G. 2 CONTINUED. 12/99

```
  MBOII
  .BSPMII
  .. MBOII
TCCGGATACGCTGGATTCAACAGCACGTCGCCAACGTCATCATCGACGGAAGGTTCCCTA
     1270      1280      1290      1300      1310      1320
 S  G  Y  A  G  F  N  S  T  S  P  T  S  S  S  T  E  G  S  L

   BSMI
   SPHI
   . MBOII
   . NSII                                  |START CE7
AGCATGCATTCCACATCTTCCAAGAGTTCAACGTCAGACGAAAAGTCTCCGTCATCAGAC
     1330      1340      1350      1360      1370      1380
 S  M  H  S  T  S  S  K  S  S  T  S  D  E  K  S  P  S  S  D

GATCTTACTCTTAACGCCTCCATCGTGACAGCTATCAGACAGCCGATAGCCGCAACACCG
     1390      1400      1410      1420      1430      1440
 D  L  T  L  N  A  S  I  V  T  A  I  R  Q  P  I  A  A  T  P

              SSPI
GTTTCTCCAAATATTATCAACAAGCCTGTTGAGGAAAAACCAACACTGGCAGTGAAAGGA
     1450      1460      1470      1480      1490      1500
 V  S  P  N  I  I  N  K  P  V  E  E  K  P  T  L  A  V  K  G

                 BINI XHOII          NSPBII
                 .    .              PVUII
GTGAAAAGCACAGCGAAAAAAGATCCACCTCCAGCTGTTCCGCCACGTGACACCCAGCCA
     1510      1520      1530      1540      1550      1560
 V  K  S  T  A  K  K  D  P  P  P  A  V  P  P  R  D  T  Q  P

                                          HINCII          ECORV
ACAATCGGAGTTGTTAGTCCAATTATGGCACATAAGAAGTTGACAAATGACCCCGTGATA
     1570      1580      1590      1600      1610      1620
 T  I  G  V  V  S  P  I  M  A  H  K  K  L  T  N  D  P  V  I

                                 SFANI
TCTGAAAAACCAGAACCTGAAAAGCTCCAATCAATGAGCATCGACACGACGGACGTTCCA
     1630      1640      1650      1660      1670      1680
 S  E  K  P  E  P  E  K  L  Q  S  M  S  I  D  T  T  D  V  P

CCGCTTCCACCTCTAAAATCAGTTGTTCCACTTAAAATGACTTCAATCCGACAACCACCA
     1690      1700      1710      1720      1730      1740
 P  L  P  P  L  K  S  V  V  P  L  K  M  T  S  I  R  Q  P  P

  MBOII
ACGTACGATGTTCTTCTAAAACAAGGAAAAATCACATCGCCTGTCAAGTCGTTTGGATAT
     1750      1760      1770      1780      1790      1800
 T  Y  D  V  L  L  K  Q  G  K  I  T  S  P  V  K  S  F  G  Y

 HGAI                            HGAI
 .                               . MBOII
GAGCAGTCGTCCGCGTCTGAAGACTCCATTGTGGCTCATGCGTCGGCTCAGGTGACTCCG
     1810      1820      1830      1840      1850      1860
 E  Q  S  S  A  S  E  D  S  I  V  A  H  A  S  A  Q  V  T  P

 HPHI                                               FOKI
CCGACAAAAAACTTCTGGTAATCATTCGCTGGAGAGAAGGATGGGAAAGAATAAGACATCA
     1870      1880      1890      1900      1910      1920
 P  T  K  T  S  G  N  H  S  L  E  R  R  M  G  K  N  K  T  S
```

FIG. 2 CONTINUED. 13/99

```
          NSPBII              AHAII    HGAI
GAATCCAGCGGCTACACCTCTGACGCCGGTGTTGCGATGTGCGCCAAAATGAGGGAGAAG
        1930      1940      1950      1960      1970      1980
 E  S  S  G  Y  T  S  D  A  G  V  A  M  C  A  K  M  R  E  K

                                     BSP1286
                                     HGIAI                  ASUII
CTGAAAGAATACGATGACATGACTCGTCGAGCACAGAACGGCTATCCTGACAACTTCGAA
        1990      2000      2010      2020      2030      2040
 L  K  E  Y  D  D  M  T  R  R  A  Q  N  G  Y  P  D  N  F  E

            MBOII                                 BANII
            .                                     BSP1286
            .                                     HGIAI
            .                                     SACI
GACAGTTCCTCCTTGTCGTCTGGAATATCCGATAACAACGAGCTCGACGACATATCCACG
        2050      2060      2070      2080      2090      2100
 D  S  S  S  L  S  S  G  I  S  D  N  N  E  L  D  D  I  S  T

                BSPMII
                .  ACCI                                   FOKI
GACGATTTGTCCGGAGTAGACATGGCAACAGTCGCCTCCAAACATAGCGACTATTCCCAC
        2110      2120      2130      2140      2150      2160
 D  D  L  S  G  V  D  M  A  T  V  A  S  K  H  S  D  Y  S  H

             MBOII
             .  MBOII                  AVAI           AVAII
TTTGTTCGCCATCCCACGTCTTCTTCCTCAAAGCCCCGAGTCCCCAGTCGGTCCTCCACA
        2170      2180      2190      2200      2210      2220
 F  V  R  H  P  T  S  S  S  S  K  P  R  V  P  S  R  S  S  T

                   AVAI
                   XHOI
TCAGTCGATTCTCGATCTCGAGCAGAACAGGAGAATGTGTACAAACTTCTGTCCCAGTGC
        2230      2240      2250      2260      2270      2280
 S  V  D  S  R  S  R  A  E  Q  E  N  V  Y  K  L  L  S  Q  C

           BBVI BGLI
           .    . BANI
           .    . .AHAII
           .    . .NARI
           .    . ..  BAEII
           .    . ..  . NSPBII                   BINI XHOII
           .    . ..  . .                         .    . FOKI
CGAACGAGCCAACGTGGCGCCGCTGCCACCTCAACCTTCGGACAACATTCGCTAAGATCC
        2290      2300      2310      2320      2330      2340
 R  T  S  Q  R  G  A  A  A  T  S  T  F  G  Q  H  S  L  R  S

AVAI
.NCII
..NCII
..SMAI                                    NSPBII
...                                       PVUII
CCGGGATACTCATCCTATTCTCCACACTTATCAGTGTCAGCTGATAAGGACACAATGTCT
        2350      2360      2370      2380      2390      2400
 P  G  Y  S  S  Y  S  P  H  L  S  V  S  A  D  K  D  T  M  S
```

FIG. 2 CONTINUED. 14/99

```
                    SPEI
                    .   SALI
                    .   .ACCI
                    .   ..HINCII
                    .   ...MBOII
ATGCACTCACAGACTAGTCGACGACCTTCTTCACAAAAACCAAGCTATTCAGGCCAATTT
        2410      2420      2430      2440      2450      2460
 M  H  S  Q  T  S  R  R  P  S  S  Q  K  P  S  Y  S  G  Q  F

                                FOKI                      BSP1286
                                .                         HGIAI
CATTCACTTGATCGTAAATGCCACCTTCAAGAGTTCACATCCACCGAGCACAGAATGGCG
        2470      2480      2490      2500      2510      2520
 H  S  L  D  R  K  C  H  L  Q  E  F  T  S  T  E  H  R  M  A

                 AVAI
                 .BANII
                 .BSP1286 BANI                       MBOII     BINI BAMHI
                 ..       .                          .         .    XHOII
GCTCTCTTGAGCCCGAGACGGGTGCCGAACTCGATGTCGAAATATGATTCTTCAGGATCC
        2530      2540      2550      2560      2570      2580
 A  L  L  S  P  R  R  V  P  N  S  M  S  K  Y  D  S  S  G  S

   BINI       AVAI
TACTCGGCGCGTTCCCGAGGTGGAAGCTCTACTGGTATCTATGGAGAGACGTTCCAACTG
        2590      2600      2610      2620      2630      2640
 Y  S  A  R  S  R  G  G  S  S  T  G  I  Y  G  E  T  F  Q  L

                                                        BINI BAMHI
                                                        .    XHOII
CACAGACTATCCGATGAAAAATCCCCCGCACATTCTGCCAAAAGTGAGATGGGATCCCAA
        2650      2660      2670      2680      2690      2700
 H  R  L  S  D  E  K  S  P  A  H  S  A  K  S  E  M  G  S  Q

BINI      NHEI          NDEI
.         .             .   XHOII   BINI
CTATCACTGGCTAGCACGACAGCATATGGATCTCTCAATGAGAAGTACGAACATGCTATT
        2710      2720      2730      2740      2750      2760
 L  S  L  A  S  T  T  A  Y  G  S  L  N  E  K  Y  E  H  A  I

                                           SALI
                                           .ACCI
                                           ..HINCII
CGGGACATGGCACGTGACTTGGAGTGTTACAAGAACACTGTCGACTCACTAACCAAGAAA
        2770      2780      2790      2800      2810      2820
 R  D  M  A  R  D  L  E  C  Y  K  N  T  V  D  S  L  T  K  K

                                           HINDIII
CAGGAGAACTATGGAGCATTGTTTGATCTTTTTGAGCAAAAGCTTAGAAAACTCACTCAA
        2830      2840      2850      2860      2870      2880
 Q  E  N  Y  G  A  L  F  D  L  F  E  Q  K  L  R  K  L  T  Q

    BINI
    .  CLAI                                MBOII
CACATTGATCGATCCAACTTGAAGCCTGAAGAGGCAATACGATTCAGGCAGGACATTGCT
        2890      2900      2910      2920      2930      2940
 H  I  D  R  S  N  L  K  P  E  E  A  I  R  F  R  Q  D  I  A
```

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F/G. 2 CONTINUED. 15/99

```
                 FOKI
                 . SFANI                                           HAEII
CATTTGAGGGATATTAGCAATCATCTTGCATCCAACTCAGCTCATGCTAACGAAGGCGCT
      2950      2960      2970      2980      2990      3000
 H  L  R  D  I  S  N  H  L  A  S  N  S  A  H  A  N  E  G  A

 MBOII      HPHI
 .          . HINCII  FOKI
 .          . .       .  SFANI             CLAI   CLAI
GGTGAGCTTCTTCGTCAACCATCTCTGGAATCAGTTGCATCCCATCGATCATCGATGTCA
      3010      3020      3030      3040      3050      3060
 G  E  L  L  R  Q  P  S  L  E  S  V  A  S  H  R  S  S  M  S

                   ECOB   BBVI        MBOII
                   .      .           .   BANII
                   .      .           .   BSP1286
                   .      .           .   HGIAI
                   .      .           .   SACI
TCGTCGTCGAAAAGCAGCAAGCAGGAGAAGATCAGCTTGAGCTCGTTTGGCAAGAACAAG
      3070      3080      3090      3100      3110      3120
 S  S  S  K  S  S  K  Q  E  K  I  S  L  S  S  F  G  K  N  K

   BINI BAMHI
   .    XHOII
   .    .   MBOII
   .    .   .   BINI    HPHI                       MBOII
   .    .   .   .       .                          .  MBOII
AAGAGCTGGATCCGCTCCTCACTCTCCAAGTTCACCAAGAAGAAGAACAAGAACTACGAC
      3130      3140      3150      3160      3170      3180
 K  S  W  I  R  S  S  L  S  K  F  T  K  K  K  N  K  N  Y  D

        NDEI            XHOII
        .               .BSPMII BINI
GAAGCACATATGCCATCAATTTCCGGATCTCAAGGAACTCTTGACAACATTGATGTGATT
      3190      3200      3210      3220      3230      3240
 E  A  H  M  P  S  I  S  G  S  Q  G  T  L  D  N  I  D  V  I

                 BANII
                 BSP1286
                 HGIAI
                 SACI ECOK      APALI
                 .    .         .   BSP1286
                 .    .         .   HGIAI
GAGTTGAAGCAAGAGCTCAAAGAACGCGATAGTGCACTTTACGAAGTCCGCCTTGACAAT
      3250      3260      3270      3280      3290      3300
 E  L  K  Q  E  L  K  E  R  D  S  A  L  Y  E  V  R  L  D  N

           BINI
           .BSP1286
CTGGATCGTGCCCGCGAAGTTGATGTTCTGAGGGAGACAGTGAACAAGTTGAAAACCGAG
      3310      3320      3330      3340      3350      3360
 L  D  R  A  R  E  V  D  V  L  R  E  T  V  N  K  L  K  T  E

                        HPHI            AVAII     MBOII
AACAAGCAATTAAAGAAAGAAGTGGACAAACTCACCAACGGTCCAGCCACTCGTGCTTCT
      3370      3380      3390      3400      3410      3420
 N  K  Q  L  K  K  E  V  D  K  L  T  N  G  P  A  T  R  A  S
```

FIG. 2 CONTINUED. 16/99

```
                                                                     SFANI
TCCCGCGCCTCAATTCCAGTTATCTACGACGATGAGCATGTCTATGATGCAGCGTGTAGC
      3430      3440      3450      3460      3470      3480
 S  R  A  S  I  P  V  I  Y  D  D  E  H  V  Y  D  A  A  C  S

 BBVI      MBOII      ASUII
                      .BINI
                      .. BBVI
AGTACATCAGCTAGTCAATCTTCGAAACGATCCTCTGGCTGCAACTCAATCAAGGTTACT
      3490      3500      3510      3520      3530      3540
 S  T  S  A  S  Q  S  S  K  R  S  S  G  C  N  S  I  K  V  T

                                    PVUI
                                        HINCII
                                        HPAI
                                            NCII
GTAAACGTGGACATCGCTGGAGAAATCAGTTCGATCGTTAACCCGGACAAAGAGATAATC
      3550      3560      3570      3580      3590      3600
 V  N  V  D  I  A  G  E  I  S  S  I  V  N  P  D  K  E  I  I

       ECORV        HINCII
GTAGGATATCTTGCCATGTCAACCAGTCAGTCATGCTGGAAAGACATTGATGTTTCTATT
      3610      3620      3630      3640      3650      3660
 V  G  Y  L  A  M  S  T  S  Q  S  C  W  K  D  I  D  V  S  I

                  ACCI           SFANI                       CLAI
CTAGGACTATTTGAAGTCTACCTATCCAGAATTGATGTGGAGCATCAACTTGGAATCGAT
      3670      3680      3690      3700      3710      3720
 L  G  L  F  E  V  Y  L  S  R  I  D  V  E  H  Q  L  G  I  D

       SFANI   STYI                HGAI     AFLIII
                                            MLUI
                                            .HPHI   HGAI
GCTCGTGATTCTATCCTTGGCTATCAAATTGGTGAACTTCGACGCGTCATTGGAGACTCC
      3730      3740      3750      3760      3770      3780
 A  R  D  S  I  L  G  Y  Q  I  G  E  L  R  R  V  I  G  D  S

    FOKI
ACAACCATGATAACCAGCCATCCAACTGACATTCTTACTTCCTCAACTACAATCCGAATG
      3790      3800      3810      3820      3830      3840
 T  T  M  I  T  S  H  P  T  D  I  L  T  S  S  T  T  I  R  M

         BANI                 ACCI      AVAII  MBOII
TTCATGCACGGTGCCGCACAGAGTCGCGTAGACAGTCTGGTCCTTGATATGCTTCTTCCA
      3850      3860      3870      3880      3890      3900
 F  M  H  G  A  A  Q  S  R  V  D  S  L  V  L  D  M  L  L  P

                                                  AHAII
                                                  . AATII
AAGCAAATGATTCTCCAACTCGTCAAGTCAATTTTGACAGAGAGACGTCTGGTGTTAGCT
      3910      3920      3930      3940      3950      3960
 K  Q  M  I  L  Q  L  V  K  S  I  L  T  E  R  R  L  V  L  A

                              BBVI     BSTNI
                                           MBOII
GGAGCAACTGGAATTGGAAAGAGCAAACTGGCGAAGACCCTGGCTGCTTATGTATCTATT
      3970      3980      3990      4000      4010      4020
 G  A  T  G  I  G  K  S  K  L  A  K  T  L  A  A  Y  V  S  I
```

FIG. 2 CONTINUED. 17/99

```
ASUII   |CE6                 MBOII    BSMI
CGAACAAATCAATCCGAAGATAGTATTGTTAATATCAGCATTCCTGAAAACAATAAAGAA
      4030      4040      4050      4060      4070      4080
 R  T  N  Q  S  E  D  S  I  V  N  I  S  I  P  E  N  N  K  E

     XMNI MBOII      AHAII
     .    .          .   BSTNI
     .    .          .   .   HGAI
     .    .          .   .   .  BGLII
     .    .          .   .   .  XHOII        SFANI       NSII
GAATTGCTTCAAGTGGAACGACGCCTGGAAAAGATCTTGAGAAGCAAAGAATCATGCATC
      4090      4100      4110      4120      4130      4140
 E  L  L  Q  V  E  R  R  L  E  K  I  L  R  S  K  E  S  C  I

      XBAI
GTAATTCTAGATAATATCCCAAAGAATCGAATTGCATTTGTTGTATCCGTTTTTGCAAAT
      4150      4160      4170      4180      4190      4200
 V  I  L  D  N  I  P  K  N  R  I  A  F  V  V  S  V  F  A  N

                      AVAII                 HINCII ECORV
GTCCCACTTCAAAACAACGAAGGTCCATTTGTAGTATGCACAGTCAACCGATATCAAATC
      4210      4220      4230      4240      4250      4260
 V  P  L  Q  N  N  E  G  P  F  V  V  C  T  V  N  R  Y  Q  I

       HPHI                                     FOKI
CCTGAGCTTCAAATTCACCACAATTTCAAAATGTCAGTAATGTCGAATCGTCTCGAAGGA
      4270      4280      4290      4300      4310      4320
 P  E  L  Q  I  H  H  N  F  K  M  S  V  M  S  N  R  L  E  G

                                                FOKI
TTCATCCTACGTTACCTCCGACGACGGGCGGTAGAGGATGAGTATCGTCTAACTGTACAG
      4330      4340      4350      4360      4370      4380
 F  I  L  R  Y  L  R  R  R  A  V  E  D  E  Y  R  L  T  V  Q

      MBOII
      .  SFANI
      .  .    BANII
      .  .    BSP1286
      .  .    HGIAI
      .  .    SACI      MBOII       MBOII
ATGCCATCAGAGCTCTTCAAAATCATTGACTTCTTCCCAATAGCTCTTCAGGCCGTCAAT
      4390      4400      4410      4420      4430      4440
 M  P  S  E  L  F  K  I  I  D  F  F  P  I  A  L  Q  A  V  N
                  ECOR1 USED FOR EXPRESSION
                  ECORI                AVAII        SPHI
AATTTTATTGAGAAAACGAATTCTGTTGATGTGACAGTTGGTCCAAGAGCATGCTTGAAC
      4450      4460      4470      4480      4490      4500
 N  F  I  E  K  T  N  S  V  D  V  T  V  G  P  R  A  C  L  N

              BINI BAMHI
              .    XHOII  BINI
TGTCCTCTAACTGTCGATGGATCCCGTGAATGGTTCATTCGATTGTGGAATGAGAACTTC
      4510      4520      4530      4540      4550      4560
 C  P  L  T  V  D  G  S  R  E  W  F  I  R  L  W  N  E  N  F

               AFLIII               BBVI
ATTCCATATTTGGAACGTGTTGCTAGAGATGGCAAAAAAAACCTTCGGTCGCTGCACTTC
                                 AAAAAA-ACC...
      4570      4580      4590      4600      4610      4620
 I  P  Y  L  E  R  V  A  R  D  G  K  K  N  L  R  S  L  H  F
                                        T  F  G  R  C  T  S
```

18/99

FIG. 2 CONTINUED.

```
BINI BAMHI
.     XHOII     BINI TTHIIII        EAEI                      NCII
CTTCGAGGATCCCACCGACATCGTCTCTAAAAAATGGCCGTGGTTCGATGGTGAAAACCC
        4630      4640      4650      4660      4670      4680
L  R  G  S  H  R  H  R  L  *
 F  E  D  P  T  D  I  V  S  E  K  W  P  W  F  D  G  E  N  P
 HPHI       MBOII
 .          .BSP1286
 .          .HGIAI                  TTHIIII
 .          ..                      .HPHI    FOKI       BSPMI
GGAGAATGTGCTCAAACGTCTTCAACTCCAAGACCTCGTCCCGTCACCTGCCAACTCATC
        4690      4700      4710      4720      4730      4740

 E  N  V  L  K  R  L  Q  L  Q  D  L  V  P  S  P  A  N  S  S

                  AVAI
                  XHOI BINI               SFANI
                  .    .                  . SPHI
CCGACAACACTTCAATCCCCTCGAGTCGTTGATCCAATTGCATGCTACCAAGCATCAGAC

        4750      4760      4770      4780      4790      4800
 R  Q  H  F  N  P  L  E  S  L  I  Q  L  H  A  T  K  H  Q  T
                     MBOII    MBOII                 MBOII
CATCGACAACATTTGAACAGAAGACTCTAATCTTCTCTCGCCTCTCCCCCGCTTTCCTTA
        4810      4820      4830      4840      4850      4860

 I  D  N  I  *
          BANI
          .   KPNI
TCTTCGTACCGGTACCTGATGATTCCCCATTTTCCCCCTTTTCCCCCCAATTTCCCAGAA
        4870      4880      4890      4900      4910      4920

                            AVAI
                            .NCII
                            ..NCII
                            ..SMAI
                            ... BANI    AHAII   HGAI  DRAI
CCTCCTGTTCCCTTTGTTCCTAGTCCTCCCGGGTGCCGACGCCGAAGCGATTTAAAAACC
        4930      4940      4950      4960      4970      4980

                XMNI
TTTTTCTTTCCGAAACATTTCCCATTGCTCATTAATAGTCAAATTGAATAAACAGTGTAT
        4990      5000      5010      5020      5030      5040

GTACTTAAAAAAAAAAAAAAAAAAAAAAAAAAA
        5050      5060      5070
```

FIG. 3. 19/99

Sequences of low complexity in UNC-53 TB3-M5 identified with the FILTER and SEG algorithms of the BLAST sequence homology package.

```
MTTSNVELIPIYTDWANRHLSKGSLSKSIRDISNDFRDYRLVSQLINVIVPINEFSPAFT
KRLAKITSNLDGLETCLDYLKNLGLDCSKLTKTDIDSGNLGAVLQLLFLLSTYXXXXXXX
XXXXXXXXXXPTSIMPPAVSKLXXXXXXXXXXXXXXXXXXXFPQMSTSRLQTPQXXXXXX
XXXXXXXXXXTSGLKPXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXNLNRPTSQLQKPSRPQTQLVRVATTTKIGSSKLAAPKAVSTPKLASVKTIGAKQEPD
NSXXXXXXMXXXXXXXXXXXXXXXXXXQPTRKAAAVPQQQTLSKIAAPVKSGLKPPTSKL
GSATSMSKLCTPKVSYRKTDAPIISQQDSKRCSKXXXXXXGYAGFNXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXDDLTLNASIVTAIRQPIAATPVSPNIINKPVEEKPTLAVKGV
KSTAKKDPPPAVPPRDTQPTIGVVSPIMAHKKLTNDPVISEKPEPEKLQSMSIDTTDXXX
XXXXXXXXXXXMTSIRQPPTYDVLLKQGKITSPVKSFGYEQSSASEDSIVAHASAQVTPP
TKTSGNHSLERRMGKNKTSESSGYTSDAGVAMCAKMREKLKEYDDMTRRAQNGYPDNFED
XXXXXXXXXDNNELDDISTDDLSGVDMATVASKHSDYSHFVRHPXXXXXXXXXXXXXXXX
XXXXXXAEQENVYKLLSQCRTSQRGAAATSTFGQHSLRSPGYSSYSPHLSVSADKDTMSM
HSQTSRRPSSQKPSYSGQFHSLDRKCHLQEFTSTEHRMAALLSPRRVPNXXXXXXXXXXX
XXXXXXXXXXXIYGETFQLHRLSDEKSPAHSAKSEMGSQLSLASTTAYGSLNEKYEHAIR
DMARDLECYKNTVDSLTKKQENYGALFDLFEQKLRKLTQHIDRSNLKPEEAIRFRQDIAH
LRDISNHLASNSAHANEGAGELLRQPSLEXXXXXXXXXXXXXXXXXXXXXXXXXFGKNKK
SWIRSSLSKFTKKKNKNYDEAHMPSISGSQGTLDNIDVIELKQELKERDSALYEVRLDNL
DRAREVDVLRETVNKLKTENKQLKKEVDKLTNGPATRASSRASIPVIYDDEHVYDXXXXX
XXXXXXXXXXXGCNXXXXXXXXXXXXXXXXXXXXDKEIIVGYLAMSTSQSCWKDIDVSIL
GLFEVYLSRIDVEHQLGIDARDSILGYQIGELRRVIGDSTTMITSHPTDILTSSTTIRMF
MHGAAQSRVDSLVLDMLLPKQMILQLVKSILTERRLVLAGATGIGKSKLAKTLAAYVSIR
TNQSEDSIVNISIPENNKEELLQVERRLEKILRSKESCIVILDNIPKNRIAFVVSVFANV
PLQNNEGPFVVCTVNRYQIPELQIHHNFKMSVMSNRLEGFILRYLRRRAVEDEYRLTVQM
PSELFKIIDFFPIALQAVNNFIEKTNSVDVTVGPRACLNCPLTVDGSREWFIRLWNENFI
PYLERVARDGKKNLRSLHFLRGSHRHRL
```

```
MTTSNVELIPIYTDWANRHLSKGSLSKSIRDISNDFRDYRLVSQLINVIVPINEFSPAFT
KRLAKITSNLDGLETCLDYLKNLGLDCSKLTKTDIDSGNLGAVLQLLFLLSTYKQKLRQL
KKDQKKLEQLPTSIMPPAVSKLPSPRVATSATASATNPNSNFPQMSTSRLQTPQSRISKI
DSSKIGIKPKTSGLKPPSSSTTSSNNTNSFRPSSRSSGNNNVGSTISTSAKSLESSSTYS
SISNLNRPTSQLQKPSRPQTQLVRVATTTKIGSSKLAAPKAVSTPKLASVKTIGAKQEPD
NSGGGGGGMLKLKLFSSKNPSSSSNSPQPTRKAAAVPQQQTLSKIAAPVKSGLKPPTSKL
GSATSMSKLCTPKVSYRKTDAPIISQQDSKRCSKSSEEESGYAGFNSTSPTSSSTEGSLS
MHSTSSKSSTSDEKSPSSDDLTLNASIVTAIRQPIAATPVSPNIINKPVEEKPTLAVKGV
KSTAKKDPPPAVPPRDTQPTIGVVSPIMAHKKLTNDPVISEKPEPEKLQSMSIDTTDVPP
LPPLKSVVPLKMTSIRQPPTYDVLLKQGKITSPVKSFGYEQSSASEDSIVAHASAQVTPP
TKTSGNHSLERRMGKNKTSESSGYTSDAGVAMCAKMREKLKEYDDMTRRAQNGYPDNFED
SSSLSSGISDNNELDDISTDDLSGVDMATVASKHSDYSHFVRHPTSSSSKPRVPSRSSTS
VDSRSRAEQENVYKLLSQCRTSQRGAAATSTFGQHSLRSPGYSSYSPHLSVSADKDTMSM
HSQTSRRPSSQKPSYSGQFHSLDRKCHLQEFTSTEHRMAALLSPRRVPNSMSKYDSSGSY
SARSRGGSSTGIYGETFQLHRLSDEKSPAHSAKSEMGSQLSLASTTAYGSLNEKYEHAIR
DMARDLECYKNTVDSLTKKQENYGALFDLFEQKLRKLTQHIDRSNLKPEEAIRFRQDIAH
LRDISNHLASNSAHANEGAGELLRQPSLESVASHRSSMSSSSKSSKQEKISLSSFGKNKK
SWIRSSLSKFTKKKNKNYDEAHMPSISGSQGTLDNIDVIELKQELKERDSALYEVRLDNL
DRAREVDVLRETVNKLKTENKQLKKEVDKLTNGPATRASSRASIPVIYDDEHVYDAACSS
```

FIG. 3 CONTINUED.

TSASQSSKRSSGCNSIKVTVNVDIAGEISSIVNPDKEIIVGYLAMSTSQSCWKDIDVSIL
GLFEVYLSRIDVEHQLGIDARDSILGYQIGELRRVIGDSTTMITSHPTDILTSSTTIRMF
MHGAAQSRVDSLVLDMLLPKQMILQLVKSILTERRLVLAGATGIGKSKLAKTLAAYVSIR
TNQSEDSIVNISIPENNKEELLQVERRLEKILRSKESCIVILDNIPKNRIAFVVSVFANV
PLQNNEGPFVVCTVNRYQIPELQIHHNFKMSVMSNRLEGFILRYLRRRAVEDEYRLTVQM
PSELFKIIDFFPIALQAVNNFIEKTNSVDVTVGPRACLNCPLTVDGSREWFIRLWNENFI
PYLERVARDGKKNLRSLHFLRGSHRHRL

FIG. 4 21/99

Length of tb3-m5.pro from cDNA pTB54 : 1528 aa; +1 at: 1;
Listed (Ordinary) from: 1 to: 1528; din, 23 apr 1996 11:49

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp 15

Ala Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg 30

Asp Ile Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln Leu 45

Ile Asn Val Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr 60

Lys Arg Leu Ala Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr 75

Cys Leu Asp Tyr Leu Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu 90

Thr Lys Thr Asp Ile Asp Ser Gly Asn Leu Gly Ala Val Leu Gln 105

Leu Leu Phe Leu Leu Ser Thr Tyr Lys Gln Lys Leu Arg Gln Leu 120

Lys Lys Asp Gln Lys Lys Leu Glu Gln Leu Pro Thr Ser Ile Met 135

Pro Pro Ala Val Ser Lys Leu Pro Ser Pro Arg Val Ala Thr Ser 150

Ala Thr Ala Ser Ala Thr Asn Pro Asn Ser Asn Phe Pro Gln Met 165

Ser Thr Ser Arg Leu Gln Thr Pro Gln Ser Arg Ile Ser Lys Ile 180

Asp Ser Ser Lys Ile Gly Ile Lys Pro Lys Thr Ser Gly Leu Lys 195

Pro Pro Ser Ser Ser Thr Thr Ser Ser Asn Asn Thr Asn Ser Phe 210

Arg Pro Ser Ser Arg Ser Ser Gly Asn Asn Asn Val Gly Ser Thr 225

Ile Ser Thr Ser Ala Lys Ser Leu Glu Ser Ser Ser Thr Tyr Ser 240

Ser Ile Ser Asn Leu Asn Arg Pro Thr Ser Gln Leu Gln Lys Pro 255

Ser Arg Pro Gln Thr Gln Leu Val Arg Val Ala Thr Thr Thr Lys 270

Ile Gly Ser Ser Lys Leu Ala Ala Pro Lys Ala Val Ser Thr Pro 285

Lys Leu Ala Ser Val Lys Thr Ile Gly Ala Lys Gln Glu Pro Asp 300

Asn Ser Gly Gly Gly Gly Gly Gly Met Leu Lys Leu Lys Leu Phe 315

Ser Ser Lys Asn Pro Ser Ser Ser Ser Asn Ser Pro Gln Pro Thr 330

Arg Lys Ala Ala Ala Val Pro Gln Gln Gln Thr Leu Ser Lys Ile 345

Ala Ala Pro Val Lys Ser Gly Leu Lys Pro Pro Thr Ser Lys Leu 360

Gly Ser Ala Thr Ser Met Ser Lys Leu Cys Thr Pro Lys Val Ser 375

Tyr Arg Lys Thr Asp Ala Pro Ile Ile Ser Gln Gln Asp Ser Lys 390

FIG. 4 CONTINUED. 22/99

Arg	Cys	Ser	Lys	Ser	Ser	Glu	Glu	Glu	Ser	Gly	Tyr	Ala	Gly	Phe	405
Asn	Ser	Thr	Ser	Pro	Thr	Ser	Ser	Ser	Thr	Glu	Gly	Ser	Leu	Ser	420
Met	His	Ser	Thr	Ser	Ser	Lys	Ser	Ser	Thr	Ser	Asp	Glu	Lys	Ser	435
Pro	Ser	Ser	Asp	Asp	Leu	Thr	Leu	Asn	Ala	Ser	Ile	Val	Thr	Ala	450
Ile	Arg	Gln	Pro	Ile	Ala	Ala	Thr	Pro	Val	Ser	Pro	Asn	Ile	Ile	465
Asn	Lys	Pro	Val	Glu	Glu	Lys	Pro	Thr	Leu	Ala	Val	Lys	Gly	Val	480
Lys	Ser	Thr	Ala	Lys	Lys	Asp	Pro	Pro	Pro	Ala	Val	Pro	Pro	Arg	495
Asp	Thr	Gln	Pro	Thr	Ile	Gly	Val	Val	Ser	Pro	Ile	Met	Ala	His	510
Lys	Lys	Leu	Thr	Asn	Asp	Pro	Val	Ile	Ser	Glu	Lys	Pro	Glu	Pro	525
Glu	Lys	Leu	Gln	Ser	Met	Ser	Ile	Asp	Thr	Thr	Asp	Val	Pro	Pro	540
Leu	Pro	Pro	Leu	Lys	Ser	Val	Val	Pro	Leu	Lys	Met	Thr	Ser	Ile	555
Arg	Gln	Pro	Pro	Thr	Tyr	Asp	Val	Leu	Leu	Lys	Gln	Gly	Lys	Ile	570
Thr	Ser	Pro	Val	Lys	Ser	Phe	Gly	Tyr	Glu	Gln	Ser	Ser	Ala	Ser	585
Glu	Asp	Ser	Ile	Val	Ala	His	Ala	Ser	Ala	Gln	Val	Thr	Pro	Pro	600
Thr	Lys	Thr	Ser	Gly	Asn	His	Ser	Leu	Glu	Arg	Arg	Met	Gly	Lys	615
Asn	Lys	Thr	Ser	Glu	Ser	Ser	Gly	Tyr	Thr	Ser	Asp	Ala	Gly	Val	630
Ala	Met	Cys	Ala	Lys	Met	Arg	Glu	Lys	Leu	Lys	Glu	Tyr	Asp	Asp	645
Met	Thr	Arg	Arg	Ala	Gln	Asn	Gly	Tyr	Pro	Asp	Asn	Phe	Glu	Asp	660
Ser	Ser	Ser	Leu	Ser	Ser	Gly	Ile	Ser	Asp	Asn	Asn	Glu	Leu	Asp	675
Asp	Ile	Ser	Thr	Asp	Asp	Leu	Ser	Gly	Val	Asp	Met	Ala	Thr	Val	690
Ala	Ser	Lys	His	Ser	Asp	Tyr	Ser	His	Phe	Val	Arg	His	Pro	Thr	705
Ser	Ser	Ser	Ser	Lys	Pro	Arg	Val	Pro	Ser	Arg	Ser	Ser	Thr	Ser	720
Val	Asp	Ser	Arg	Ser	Arg	Ala	Glu	Gln	Glu	Asn	Val	Tyr	Lys	Leu	735
Leu	Ser	Gln	Cys	Arg	Thr	Ser	Gln	Arg	Gly	Ala	Ala	Ala	Thr	Ser	750
Thr	Phe	Gly	Gln	His	Ser	Leu	Arg	Ser	Pro	Gly	Tyr	Ser	Ser	Tyr	765

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FIG. 4 CONTINUED. 23/99

Ser Pro His Leu Ser Val Ser Ala Asp Lys Asp Thr Met Ser Met 780

His Ser Gln Thr Ser Arg Arg Pro Ser Ser Gln Lys Pro Ser Tyr 795

Ser Gly Gln Phe His Ser Leu Asp Arg Lys Cys His Leu Gln Glu 810

Phe Thr Ser Thr Glu His Arg Met Ala Ala Leu Leu Ser Pro Arg 825

Arg Val Pro Asn Ser Met Ser Lys Tyr Asp Ser Ser Gly Ser Tyr 840

Ser Ala Arg Ser Arg Gly Gly Ser Ser Thr Gly Ile Tyr Gly Glu 855

Thr Phe Gln Leu His Arg Leu Ser Asp Glu Lys Ser Pro Ala His 870

Ser Ala Lys Ser Glu Met Gly Ser Gln Leu Ser Leu Ala Ser Thr 885

Thr Ala Tyr Gly Ser Leu Asn Glu Lys Tyr Glu His Ala Ile Arg 900

Asp Met Ala Arg Asp Leu Glu Cys Tyr Lys Asn Thr Val Asp Ser 915

Leu Thr Lys Lys Gln Glu Asn Tyr Gly Ala Leu Phe Asp Leu Phe 930

Glu Gln Lys Leu Arg Lys Leu Thr Gln His Ile Asp Arg Ser Asn 945

Leu Lys Pro Glu Glu Ala Ile Arg Phe Arg Gln Asp Ile Ala His 960

Leu Arg Asp Ile Ser Asn His Leu Ala Ser Asn Ser Ala His Ala 975

Asn Glu Gly Ala Gly Glu Leu Leu Arg Gln Pro Ser Leu Glu Ser 990

Val Ala Ser His Arg Ser Ser Met Ser Ser Ser Ser Lys Ser Ser 1005

Lys Gln Glu Lys Ile Ser Leu Ser Ser Phe Gly Lys Asn Lys Lys 1020

Ser Trp Ile Arg Ser Ser Leu Ser Lys Phe Thr Lys Lys Lys Asn 1035

Lys Asn Tyr Asp Glu Ala His Met Pro Ser Ile Ser Gly Ser Gln 1050

Gly Thr Leu Asp Asn Ile Asp Val Ile Glu Leu Lys Gln Glu Leu 1065

Lys Glu Arg Asp Ser Ala Leu Tyr Glu Val Arg Leu Asp Asn Leu 1080

Asp Arg Ala Arg Glu Val Asp Val Leu Arg Glu Thr Val Asn Lys 1095

Leu Lys Thr Glu Asn Lys Gln Leu Lys Lys Glu Val Asp Lys Leu 1110

Thr Asn Gly Pro Ala Thr Arg Ala Ser Ser Arg Ala Ser Ile Pro 1125

Val Ile Tyr Asp Asp Glu His Val Tyr Asp Ala Ala Cys Ser Ser 1140

FIG. 4 CONTINUED. 24/99

Thr Ser Ala Ser Gln Ser Ser Lys Arg Ser Ser Gly Cys Asn Ser 1155

Ile Lys Val Thr Val Asn Val Asp Ile Ala Gly Glu Ile Ser Ser 1170

Ile Val Asn Pro Asp Lys Glu Ile Ile Val Gly Tyr Leu Ala Met 1185

Ser Thr Ser Gln Ser Cys Trp Lys Asp Ile Asp Val Ser Ile Leu 1200

Gly Leu Phe Glu Val Tyr Leu Ser Arg Ile Asp Val Glu His Gln 1215

Leu Gly Ile Asp Ala Arg Asp Ser Ile Leu Gly Tyr Gln Ile Gly 1230

Glu Leu Arg Arg Val Ile Gly Asp Ser Thr Thr Met Ile Thr Ser 1245

His Pro Thr Asp Ile Leu Thr Ser Ser Thr Thr Ile Arg Met Phe 1260

Met His Gly Ala Ala Gln Ser Arg Val Asp Ser Leu Val Leu Asp 1275

Met Leu Leu Pro Lys Gln Met Ile Leu Gln Leu Val Lys Ser Ile 1290

Leu Thr Glu Arg Arg Leu Val Leu Ala Gly Ala Thr Gly Ile Gly 1305

Lys Ser Lys Leu Ala Lys Thr Leu Ala Ala Tyr Val Ser Ile Arg 1320

Thr Asn Gln Ser Glu Asp Ser Ile Val Asn Ile Ser Ile Pro Glu 1335

Asn Asn Lys Glu Glu Leu Leu Gln Val Glu Arg Arg Leu Glu Lys 1350

Ile Leu Arg Ser Lys Glu Ser Cys Ile Val Ile Leu Asp Asn Ile 1365

Pro Lys Asn Arg Ile Ala Phe Val Val Ser Val Phe Ala Asn Val 1380

Pro Leu Gln Asn Asn Glu Gly Pro Phe Val Val Cys Thr Val Asn 1395

Arg Tyr Gln Ile Pro Glu Leu Gln Ile His His Asn Phe Lys Met 1410

Ser Val Met Ser Asn Arg Leu Glu Gly Phe Ile Leu Arg Tyr Leu 1425

Arg Arg Arg Ala Val Glu Asp Glu Tyr Arg Leu Thr Val Gln Met 1440

Pro Ser Glu Leu Phe Lys Ile Ile Asp Phe Phe Pro Ile Ala Leu 1455

Gln Ala Val Asn Asn Phe Ile Glu Lys Thr Asn Ser Val Asp Val 1470

Thr Val Gly Pro Arg Ala Cys Leu Asn Cys Pro Leu Thr Val Asp 1485

Gly Ser Arg Glu Trp Phe Ile Arg Leu Trp Asn Glu Asn Phe Ile 1500

Pro Tyr Leu Glu Arg Val Ala Arg Asp Gly Lys Lys Asn Leu Arg 1515

Ser Leu His Phe Leu Arg Gly Ser His Arg His Arg Leu

FIG. 5. 25/99

Annotated sequence of 7A variant of UNC-53

```
        10         20         30         40         50         60
MTTSNVELIP IYTDWANRHL SKGSLSKSIR DISNDFRDYR LVSQLINVIV PINEFSPAFT
start tb6 and tb3 similarity to amino-termini of alfa-actinin,

        70         80         90        100        110        120
KRLAKITSNL DGLETCLDYL KNLGLDCSKL TKTDIDSGNL GAVLQLLFLL STYKQKLRQL
beta-spectrin, dystrosphin, fimbrin, filamin actin-binding site 1
                                                        (114 - 133)

       130        140        150        160        170        180
KKDQKKLEQL PTSIMPPAVS KLPSPRVATS ATASATNPNS NFPQMSTSRL QTPQSRISKI
   Start S4     poss. start tb1b & tb6 & tb1 lamda clone

       190        200        210        220        230        240
DSSKIGIKPK TSGLKPPSSS TTSSNNTNSF RPSSRSSGNN NVGSTISTSA KSLESSSTYS

       250        260        270        280        290        300
SISNLNRPTS QLQKPSRPQT QLVRVATTTK IGSSKLAAPK AVSTPKLASV KTIGAKQEPD

       310        320        330        340        350        360
NSGGGGGGML KLKLFSSKNP SSSSNSPQPT RKAAAVPQQQ TLSKIAAPVK SGLKPPTSKL

       370        380        390        400        410        420
GSATSMSKLC TPKVSYRKTD APIISQQDSK RCSKSSEEES GYAGFNSTSP TSSSTEGSLS

       430        440        450        460        470        480
MHSTSSKSST SDEKSPSSDD LTLNASIVTA IRQPIAATPV SPNIINKPVE EKPTLAVKGV
poss. start tb22
       490        500        510        520        530        540
KSTAKKDPPP AVPPRDTQPT IGVVSPIMAH KKLTNDPVIS EKPEPEKLQS MSIDTTDVPP
      SH3-binding 1                                            SH3-

       550        560        570        580        590        600
LPPLKSVVPL KMTSIRQPPT YDVLLKQGKI TSPVKSFGYE QSSASEDSIV AHASAQVTPP
binding 2

       610        620        630        640        650        660
TKTSGNHSLE RRMGKNKTSE SSGYTSDAGV AMCAKMREKL KEYDDMTRRA QNGYPDNFED

       670        680        690        700        710        720
SSSLSSGISD NNELDDISTD DLSGVDMATV ASKHSDYSHF VRHPTSSSSK PRVPSRSSTS

       730        740        750        760        770        780
VDSRSRAEQE NVYKLLSQCR TSQRGAAATS TFGQHSLRSP GYSSYSPHLS VSADKDTMSM

       790        800        810        820        830        840
HSQTSRRPSS QKPSYSGQFH SLDRKCHLQE FTSTEHRMAA LLSPRRVPNS MSKYDSSGSY
                  Kohara Exon deleted in cDNA YK25D6
```

SUBSTITUTE SHEET (RULE 26)

FIG. 5 CONTINUED.

```
         10         20         30         40         50         60         70
AALNASGMSR SMILLESLSP RPPRRHQSPA DSCIITASPS APRRSHSPRG PTARIPLSLA SSPVHVNNNW
predicted exon (alternative/additional to Kohara exon to be inserted after
aminoacid 838 )
```

```
       850        860        870        880        890        900
SARSRGGSST GIYGETFQLH RLSDEKSPAH SAKSEMGSQL SLASTTAYGS LNEKYEHAIR

       910        920        930        940        950        960
DMARDLECYK NTVDSLTKKQ ENYGALFDLF EQKLRKLTQH IDRSNLKPEE AIRFRQDIAH

       970        980        990       1000       1010       1020
LRDISNHLAS NSAHANEGAG ELLRQPSLES VASHRSSMSS SSKSSKQEKI SLSSFGKNKK

      1030       1040       1050       1060       1070       1080
SWIRSSLSKF TKKKNKNYDE AHMPSISGSQ GTLDNIDVIE LKQELKERDS ALYEVRLDNL
   candidate nuclear   Start GP45   localization signal

      1090       1100       1110       1120       1130       1140
DRAREVDVLR ETVNKLKTEN KQLKKEVDKL TNGPATRASS RASIPVIYDD EHVYDAACSS
                 actin binding site 2
                 (1097-1116)
      *     *     *     *
      canditate leucine zipper.pattern

      1150       1160       1170       1180       1190       1200
TSASQSSKRS SGCNSIKVTV NVDIAGEISS IVNPDKEIIV GYLAMSTSQS CWKDIDVSIL

      1210       1220       1230       1240       1250       1260
GLFEVYLSRI DVEHQLGIDA RDSILGYQIG ELRRVIGDST TMITSHPTDI LTSSTTIRMF

      1270       1280       1290       1300       1310       1320
MHGAAQSRVD SLVLDMLLPK QMILQLVKSI LTERRLVLAG ATGIGKSKLA KTLAAYVSIR
                 *     *     *     * nucleotide binding pocket
          canditate leucine zipper.pattern
      1330       1340       1350       1360       1370       1380
TNQSEDSIVN ISIPENNKEE LLQVERRLEK ILRSKESCIV ILDNIPKNRI AFVVSVFANV

      1390       1400       1410       1420       1430       1440
PLQNNEGPFV VCTVNRYQIP ELQIHHNFKM SVMSNRLEGF ILRYLRRRAV EDEYRLTVQM

      1450       1460       1470       1480       1490       1500
PSELFKIIDF FPIALQAVNN FIEKTNSVDV TVGPRACLNC PLTVDGSREW FIRLWNENFI
                       end GP45

      1510       1520       1530       1540       1550       1560
PYLERVARDG KKTFGRCTSF EDPTDIVSEK WPWFDGENPE NVLKRLQLQD LVPSPANSSR

      1570       1580
QHFNPLESLI QLHATKHQTI DNI
```

FIG. 6. 27/99

Length of Untitled : 1583 aa from cDNA pTB72; +1 at: 1;
Listed (Ordinary) from: 1 to: 1583; din, 23 apr 1996 11:37

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp 15

Ala Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg 30

Asp Ile Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln Leu 45

Ile Asn Val Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr 60

Lys Arg Leu Ala Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr 75

Cys Leu Asp Tyr Leu Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu 90

Thr Lys Thr Asp Ile Asp Ser Gly Asn Leu Gly Ala Val Leu Gln 105

Leu Leu Phe Leu Leu Ser Thr Tyr Lys Gln Lys Leu Arg Gln Leu 120

Lys Lys Asp Gln Lys Lys Leu Glu Gln Leu Pro Thr Ser Ile Met 135

Pro Pro Ala Val Ser Lys Leu Pro Ser Pro Arg Val Ala Thr Ser 150

Ala Thr Ala Ser Ala Thr Asn Pro Asn Ser Asn Phe Pro Gln Met 165

Ser Thr Ser Arg Leu Gln Thr Pro Gln Ser Arg Ile Ser Lys Ile 180

Asp Ser Ser Lys Ile Gly Ile Lys Pro Lys Thr Ser Gly Leu Lys 195

Pro Pro Ser Ser Ser Thr Thr Ser Ser Asn Asn Thr Asn Ser Phe 210

Arg Pro Ser Ser Arg Ser Ser Gly Asn Asn Asn Val Gly Ser Thr 225

Ile Ser Thr Ser Ala Lys Ser Leu Glu Ser Ser Ser Thr Tyr Ser 240

Ser Ile Ser Asn Leu Asn Arg Pro Thr Ser Gln Leu Gln Lys Pro 255

Ser Arg Pro Gln Thr Gln Leu Val Arg Val Ala Thr Thr Thr Lys 270

Ile Gly Ser Ser Lys Leu Ala Ala Pro Lys Ala Val Ser Thr Pro 285

Lys Leu Ala Ser Val Lys Thr Ile Gly Ala Lys Gln Glu Pro Asp 300

Asn Ser Gly Gly Gly Gly Gly Gly Met Leu Lys Leu Lys Leu Phe 315

Ser Ser Lys Asn Pro Ser Ser Ser Ser Asn Ser Pro Gln Pro Thr 330

Arg Lys Ala Ala Ala Val Pro Gln Gln Gln Thr Leu Ser Lys Ile 345

Ala Ala Pro Val Lys Ser Gly Leu Lys Pro Pro Thr Ser Lys Leu 360

Gly Ser Ala Thr Ser Met Ser Lys Leu Cys Thr Pro Lys Val Ser 375

FIG. 6 CONTINUED. 28/99

Tyr	Arg	Lys	Thr	Asp	Ala	Pro	Ile	Ile	Ser	Gln	Gln	Asp	Ser	Lys	390
Arg	Cys	Ser	Lys	Ser	Ser	Glu	Glu	Glu	Ser	Gly	Tyr	Ala	Gly	Phe	405
Asn	Ser	Thr	Ser	Pro	Thr	Ser	Ser	Ser	Thr	Glu	Gly	Ser	Leu	Ser	420
Met	His	Ser	Thr	Ser	Ser	Lys	Ser	Ser	Thr	Ser	Asp	Glu	Lys	Ser	435
Pro	Ser	Ser	Asp	Asp	Leu	Thr	Leu	Asn	Ala	Ser	Ile	Val	Thr	Ala	450
Ile	Arg	Gln	Pro	Ile	Ala	Ala	Thr	Pro	Val	Ser	Pro	Asn	Ile	Ile	465
Asn	Lys	Pro	Val	Glu	Glu	Lys	Pro	Thr	Leu	Ala	Val	Lys	Gly	Val	480
Lys	Ser	Thr	Ala	Lys	Lys	Asp	Pro	Pro	Pro	Ala	Val	Pro	Pro	Arg	495
Asp	Thr	Gln	Pro	Thr	Ile	Gly	Val	Val	Ser	Pro	Ile	Met	Ala	His	510
Lys	Lys	Leu	Thr	Asn	Asp	Pro	Val	Ile	Ser	Glu	Lys	Pro	Glu	Pro	525
Glu	Lys	Leu	Gln	Ser	Met	Ser	Ile	Asp	Thr	Thr	Asp	Val	Pro	Pro	540
Leu	Pro	Pro	Leu	Lys	Ser	Val	Val	Pro	Leu	Lys	Met	Thr	Ser	Ile	555
Arg	Gln	Pro	Pro	Thr	Tyr	Asp	Val	Leu	Leu	Lys	Gln	Gly	Lys	Ile	570
Thr	Ser	Pro	Val	Lys	Ser	Phe	Gly	Tyr	Glu	Gln	Ser	Ser	Ala	Ser	585
Glu	Asp	Ser	Ile	Val	Ala	His	Ala	Ser	Ala	Gln	Val	Thr	Pro	Pro	600
Thr	Lys	Thr	Ser	Gly	Asn	His	Ser	Leu	Glu	Arg	Arg	Met	Gly	Lys	615
Asn	Lys	Thr	Ser	Glu	Ser	Ser	Gly	Tyr	Thr	Ser	Asp	Ala	Gly	Val	630
Ala	Met	Cys	Ala	Lys	Met	Arg	Glu	Lys	Leu	Lys	Glu	Tyr	Asp	Asp	645
Met	Thr	Arg	Arg	Ala	Gln	Asn	Gly	Tyr	Pro	Asp	Asn	Phe	Glu	Asp	660
Ser	Ser	Ser	Leu	Ser	Ser	Gly	Ile	Ser	Asp	Asn	Asn	Glu	Leu	Asp	675
Asp	Ile	Ser	Thr	Asp	Asp	Leu	Ser	Gly	Val	Asp	Met	Ala	Thr	Val	690
Ala	Ser	Lys	His	Ser	Asp	Tyr	Ser	His	Phe	Val	Arg	His	Pro	Thr	705
Ser	Ser	Ser	Ser	Lys	Pro	Arg	Val	Pro	Ser	Arg	Ser	Ser	Thr	Ser	720
Val	Asp	Ser	Arg	Ser	Arg	Ala	Glu	Gln	Glu	Asn	Val	Tyr	Lys	Leu	735
Leu	Ser	Gln	Cys	Arg	Thr	Ser	Gln	Arg	Gly	Ala	Ala	Ala	Thr	Ser	750
Thr	Phe	Gly	Gln	His	Ser	Leu	Arg	Ser	Pro	Gly	Tyr	Ser	Ser	Tyr	765
Ser	Pro	His	Leu	Ser	Val	Ser	Ala	Asp	Lys	Asp	Thr	Met	Ser	Met	780

FIG. 6 CONTINUED. 29/99

His Ser Gln Thr Ser Arg Arg Pro Ser Ser Gln Lys Pro Ser Tyr 795

Ser Gly Gln Phe His Ser Leu Asp Arg Lys Cys His Leu Gln Glu 810

Phe Thr Ser Thr Glu His Arg Met Ala Ala Leu Leu Ser Pro Arg 825

Arg Val Pro Asn Ser Met Ser Lys Tyr Asp Ser Ser Gly Ser Tyr 840

Ser Ala Arg Ser Arg Gly Gly Ser Ser Thr Gly Ile Tyr Gly Glu 855

Thr Phe Gln Leu His Arg Leu Ser Asp Glu Lys Ser Pro Ala His 870

Ser Ala Lys Ser Glu Met Gly Ser Gln Leu Ser Leu Ala Ser Thr 885

Thr Ala Tyr Gly Ser Leu Asn Glu Lys Tyr Glu His Ala Ile Arg 900

Asp Met Ala Arg Asp Leu Glu Cys Tyr Lys Asn Thr Val Asp Ser 915

Leu Thr Lys Lys Gln Glu Asn Tyr Gly Ala Leu Phe Asp Leu Phe 930

Glu Gln Lys Leu Arg Lys Leu Thr Gln His Ile Asp Arg Ser Asn 945

Leu Lys Pro Glu Glu Ala Ile Arg Phe Arg Gln Asp Ile Ala His 960

Leu Arg Asp Ile Ser Asn His Leu Ala Ser Asn Ser Ala His Ala 975

Asn Glu Gly Ala Gly Glu Leu Leu Arg Gln Pro Ser Leu Glu Ser 990

Val Ala Ser His Arg Ser Ser Met Ser Ser Ser Ser Lys Ser Ser 1005

Lys Gln Glu Lys Ile Ser Leu Ser Ser Phe Gly Lys Asn Lys Lys 1020

Ser Trp Ile Arg Ser Ser Leu Ser Lys Phe Thr Lys Lys Lys Asn 1035

Lys Asn Tyr Asp Glu Ala His Met Pro Ser Ile Ser Gly Ser Gln 1050

Gly Thr Leu Asp Asn Ile Asp Val Ile Glu Leu Lys Gln Glu Leu 1065

Lys Glu Arg Asp Ser Ala Leu Tyr Glu Val Arg Leu Asp Asn Leu 1080

Asp Arg Ala Arg Glu Val Asp Val Leu Arg Glu Thr Val Asn Lys 1095

Leu Lys Thr Glu Asn Lys Gln Leu Lys Lys Glu Val Asp Lys Leu 1110

Thr Asn Gly Pro Ala Thr Arg Ala Ser Ser Arg Ala Ser Ile Pro 1125

Val Ile Tyr Asp Asp Glu His Val Tyr Asp Ala Ala Cys Ser Ser 1140

Thr Ser Ala Ser Gln Ser Ser Lys Arg Ser Ser Gly Cys Asn Ser 1155,

Ile Lys Val Thr Val Asn Val Asp Ile Ala Gly Glu Ile Ser Ser 1170

Ile Val Asn Pro Asp Lys Glu Ile Ile Val Gly Tyr Leu Ala Met 1185

FIG. 6 CONTINUED. 30/99

Ser Thr Ser Gln Ser Cys Trp Lys Asp Ile Asp Val Ser Ile Leu 1200

Gly Leu Phe Glu Val Tyr Leu Ser Arg Ile Asp Val Glu His Gln 1215

Leu Gly Ile Asp Ala Arg Asp Ser Ile Leu Gly Tyr Gln Ile Gly 1230

Glu Leu Arg Arg Val Ile Gly Asp Ser Thr Thr Met Ile Thr Ser 1245

His Pro Thr Asp Ile Leu Thr Ser Ser Thr Thr Ile Arg Met Phe 1260

Met His Gly Ala Ala Gln Ser Arg Val Asp Ser Leu Val Leu Asp 1275

Met Leu Leu Pro Lys Gln Met Ile Leu Gln Leu Val Lys Ser Ile 1290

Leu Thr Glu Arg Arg Leu Val Leu Ala Gly Ala Thr Gly Ile Gly 1305

Lys Ser Lys Leu Ala Lys Thr Leu Ala Ala Tyr Val Ser Ile Arg 1320

Thr Asn Gln Ser Glu Asp Ser Ile Val Asn Ile Ser Ile Pro Glu 1335

Asn Asn Lys Glu Glu Leu Leu Gln Val Glu Arg Arg Leu Glu Lys 1350

Ile Leu Arg Ser Lys Glu Ser Cys Ile Val Ile Leu Asp Asn Ile 1365

Pro Lys Asn Arg Ile Ala Phe Val Val Ser Val Phe Ala Asn Val 1380

Pro Leu Gln Asn Asn Glu Gly Pro Phe Val Val Cys Thr Val Asn 1395

Arg Tyr Gln Ile Pro Glu Leu Gln Ile His His Asn Phe Lys Met 1410

Ser Val Met Ser Asn Arg Leu Glu Gly Phe Ile Leu Arg Tyr Leu 1425

Arg Arg Arg Ala Val Glu Asp Glu Tyr Arg Leu Thr Val Gln Met 1440

Pro Ser Glu Leu Phe Lys Ile Ile Asp Phe Phe Pro Ile Ala Leu 1455

Gln Ala Val Asn Asn Phe Ile Glu Lys Thr Asn Ser Val Asp Val 1470

Thr Val Gly Pro Arg Ala Cys Leu Asn Cys Pro Leu Thr Val Asp 1485

Gly Ser Arg Glu Trp Phe Ile Arg Leu Trp Asn Glu Asn Phe Ile 1500

Pro Tyr Leu Glu Arg Val Ala Arg Asp Gly Lys Lys Thr Phe Gly 1515

Arg Cys Thr Ser Phe Glu Asp Pro Thr Asp Ile Val Ser Lys Lys 1530

Trp Pro Trp Phe Asp Gly Glu Asn Pro Glu Asn Val Leu Lys Arg 1545

Leu Gln Leu Gln Asp Leu Val Pro Ser Pro Ala Asn Ser Ser Arg 1560

Gln His Phe Asn Pro Leu Glu Ser Leu Ile Gln Leu His Ala Thr 1575

Lys His Gln Thr Ile Asp Asn Ile .

FIG. 7. 31/99

MTTSNVELIPIYTDWANRHLSKGSLSKSIRDISNDFRDYRLVSQLINVIVPINEFSPAFT
KRLAKITSNLDGLETCLDYLKNLGLDCSKLTKTDIDSGNLGAVLQLLFLLSTYXXXXXXX
XXXXXXXXXXPTSIMPPAVSKLXXXXXXXXXXXXXXXXXXXFPQMSTSRLQTPQXXXXXX
XXXXXXXXXXTSGLKPXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
XXXNLNRPTSQLQKPSRPQTQLVRVATTTKIGSSKLAAPKAVSTPKLASVKTIGAKQEPD
NSXXXXXXMXXXXXXXXXXXXXXXXXXQPTRKAAAVPQQQTLSKIAAPVKSGLKPPTSKL
GSATSMSKLCTPKVSYRKTDAPIISQQDSKRCSKXXXXXXGYAGFNXXXXXXXXXXXXXX
XXXXXXXXXXXXXXXXXXDDLTLNASIVTAIRQPIAATPVSPNIINKPVEEKPTLAVKGV
KSTAKKDPPPAVPPRDTQPTIGVVSPIMAHKKLTNDPVISEKPEPEKLQSMSIDTTDXXX
XXXXXXXXXXXMTSIRQPPTYDVLLKQGKITSPVKSFGYEQSSASEDSIVAHASAQVTPP
TKTSGNHSLERRMGKNKTSESSGYTSDAGVAMCAKMREKLKEYDDMTRRAQNGYPDNFED
XXXXXXXXXDNNELDDISTDDLSGVDMATVASKHSDYSHFVRHPXXXXXXXXXXXXXXXX
XXXXXXAEQENVYKLLSQCRTSQRGAAATSTFGQHSLRSPGYSSYSPHLSVSADKDTMSM
HSQTSRRPSSQKPSYSGQFHSLDRKCHLQEFTSTEHRMAALLSPRRVPNXXXXXXXXXXX
XXXXXXXXXXXIYGETFQLHRLSDEKSPAHSAKSEMGSQLSLASTTAYGSLNEKYEHAIR
DMARDLECYKNTVDSLTKKQENYGALFDLFEQKLRKLTQHIDRSNLKPEEAIRFRQDIAH
LRDISNHLASNSAHANEGAGELLRQPSLEXXXXXXXXXXXXXXXXXXXXXXXXXFGKNKK
SWIRSSLSKFTKKKNKNYDEAHMPSISGSQGTLDNIDVIELKQELKERDSALYEVRLDNL
DRAREVDVLRETVNKLKTENKQLKKEVDKLTNGPATRASSRASIPVIYDDEHVYDXXXXX
XXXXXXXXXXXGCNXXXXXXXXXXXXXXXXXXXXDKEIIVGYLAMSTSQSCWKDIDVSIL
GLFEVYLSRIDVEHQLGIDARDSILGYQIGELRRVIGDSTTMITSHPTDILTSSTTIRMF
MHGAAQSRVDSLVLDMLLPKQMILQLVKSILTERRLVLAGATGIGKSKLAKTLAAYVSIR
TNQSEDSIVNISIPENNKEELLQVERRLEKILRSKESCIVILDNIPKNRIAFVVSVFANV
PLQNNEGPFVVCTVNRYQIPELQIHHNFKMSVMSNRLEGFILRYLRRRAVEDEYRLTVQM
PSELFKIIDFFPIALQAVNNFIEKTNSVDVTVGPRACLNCPLTVDGSREWFIRLWNENFI
PYLERVARDGKKNLRSLHFLRGSHRHRL

MTTSNVELIPIYTDWANRHLSKGSLSKSIRDISNDFRDYRLVSQLINVIVPINEFSPAFT
KRLAKITSNLDGLETCLDYLKNLGLDCSKLTKTDIDSGNLGAVLQLLFLLSTYKQKLRQL
KKDQKKLEQLPTSIMPPAVSKLPSPRVATSATASATNPNSNFPQMSTSRLQTPQSRISKI
DSSKIGIKPKTSGLKPPSSSTTSSNNTNSFRPSSRSSGNNNVGSTISTSAKSLESSSTYS
SISNLNRPTSQLQKPSRPQTQLVRVATTTKIGSSKLAAPKAVSTPKLASVKTIGAKQEPD
NSGGGGGGMLKLKLFSSKNPSSSSNSPQPTRKAAAVPQQQTLSKIAAPVKSGLKPPTSKL
GSATSMSKLCTPKVSYRKTDAPIISQQDSKRCSKSSEEESGYAGFNSTSPTSSSTEGSLS
MHSTSSKSSTSDEKSPSSDDLTLNASIVTAIRQPIAATPVSPNIINKPVEEKPTLAVKGV
KSTAKKDPPPAVPPRDTQPTIGVVSPIMAHKKLTNDPVISEKPEPEKLQSMSIDTTDVPP
LPPLKSVVPLKMTSIRQPPTYDVLLKQGKITSPVKSFGYEQSSASEDSIVAHASAQVTPP
TKTSGNHSLERRMGKNKTSESSGYTSDAGVAMCAKMREKLKEYDDMTRRAQNGYPDNFED
SSSLSSGISDNNELDDISTDDLSGVDMATVASKHSDYSHFVRHPTSSSSKPRVPSRSSTS
VDSRSRAEQENVYKLLSQCRTSQRGAAATSTFGQHSLRSPGYSSYSPHLSVSADKDTMSM
HSQTSRRPSSQKPSYSGQFHSLDRKCHLQEFTSTEHRMAALLSPRRVPNSMSKYDSSGSY
SARSRGGSSTGIYGETFQLHRLSDEKSPAHSAKSEMGSQLSLASTTAYGSLNEKYEHAIR
DMARDLECYKNTVDSLTKKQENYGALFDLFEQKLRKLTQHIDRSNLKPEEAIRFRQDIAH
LRDISNHLASNSAHANEGAGELLRQPSLESVASHRSSMSSSSKSSKQEKISLSSFGKNKK
SWIRSSLSKFTKKKNKNYDEAHMPSISGSQGTLDNIDVIELKQELKERDSALYEVRLDNL
DRAREVDVLRETVNKLKTENKQLKKEVDKLTNGPATRASSRASIPVIYDDEHVYDAACSS

FIG. 7 CONTINUED.

<u>TSASQSSKRSS</u>GCN<u>SIKVTVNVDIAGEISSIVNP</u>DKEIIVGYLAMSTSQSCWKDIDVSIL
GLFEVYLSRIDVEHQLGIDARDSILGYQIGELRRVIGDSTTMITSHPTDILTSSTTIRMF
MHGAAQSRVDSLVLDMLLPKQMILQLVKSILTERRLVLAGATGIGKSKLAKTLAAYVSIR
TNQSEDSIVNISIPENNKEELLQVERRLEKILRSKESCIVILDNIPKNRIAFVVSVFANV
PLQNNEGPFVVCTVNRYQIPELQIHHNFKMSVMSNRLEGFILRYLRRRAVEDEYRLTVQM
PSELFKIIDFFPIALQAVNNFIEKTNSVDVTVGPRACLNCPLTVDGSREWFIRLWNENFI
PYLERVARDGKKNLRSLHFLRGSHRHRL

33/99

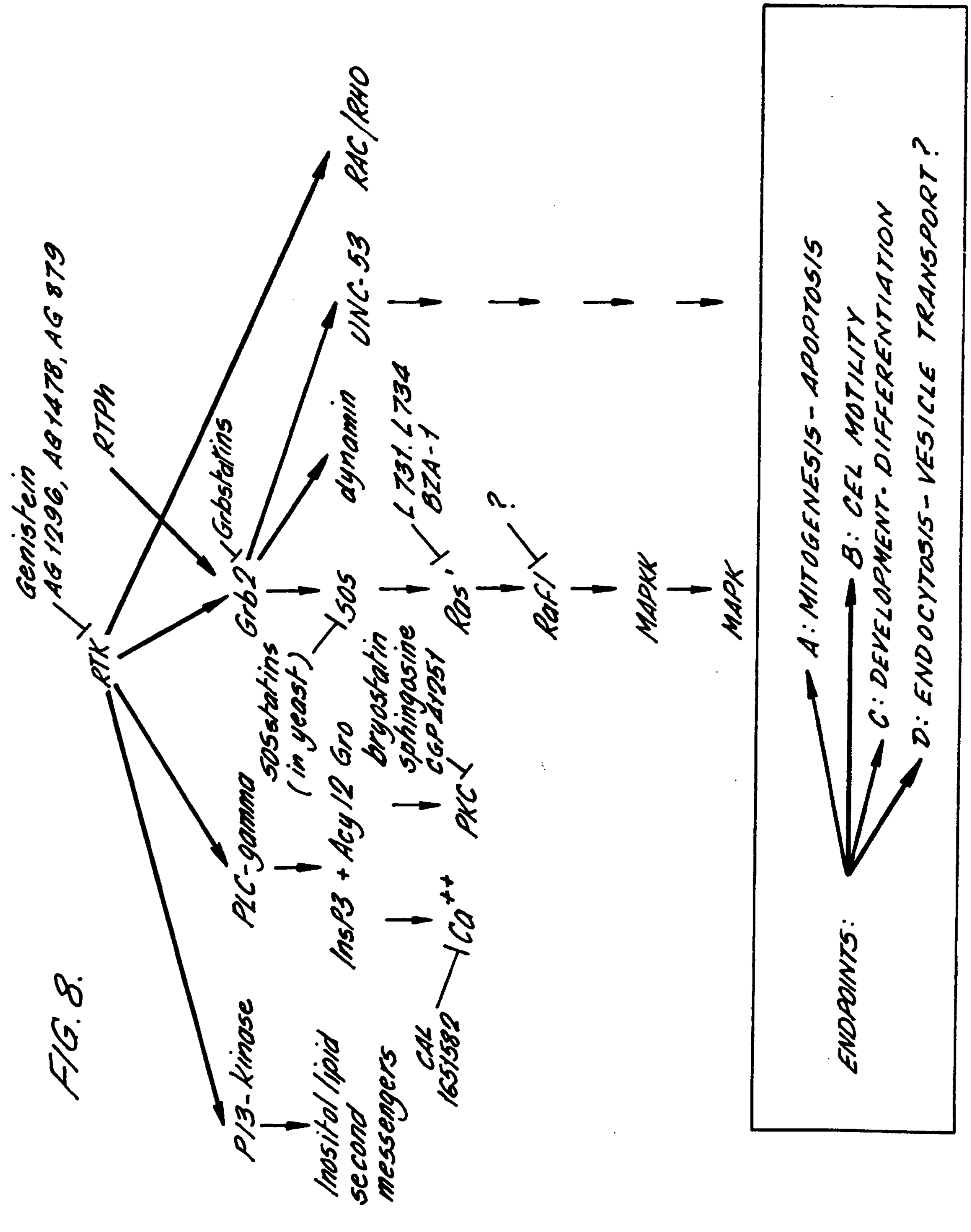
Genistein
AG 1296, AB 1478, AG 879
RTPh
Grbstatins
RTK
Grb2
SOS
Ras
Raf1
MAPKK
MAPK
dynamin
UNC-53
RAC/RHO
L 731. L 734
BZA-1
?
SOSstatins
(in yeast)
PLC-gamma
InsP3 + Acyl2 Gro
bryostatin
sphingosine
CGP41251
PKC
Ca++
CAL
165582
PI3-kinase
Inositol lipid
second
messengers
ENDPOINTS:
A: MITOGENESIS - APOPTOSIS
B: CEL MOTILITY
C: DEVELOPMENT - DIFFERENTIATION
D: ENDOCYTOSIS - VESICLE TRANSPORT ?

FIG. 8.

FIG. 9.

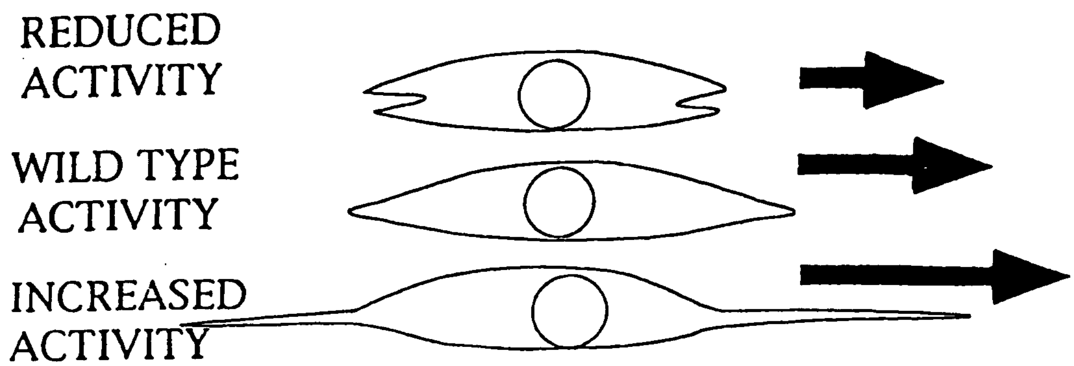
REDUCED
ACTIVITY
WILD TYPE
ACTIVITY
INCREASED
ACTIVITY

FIG. 10.

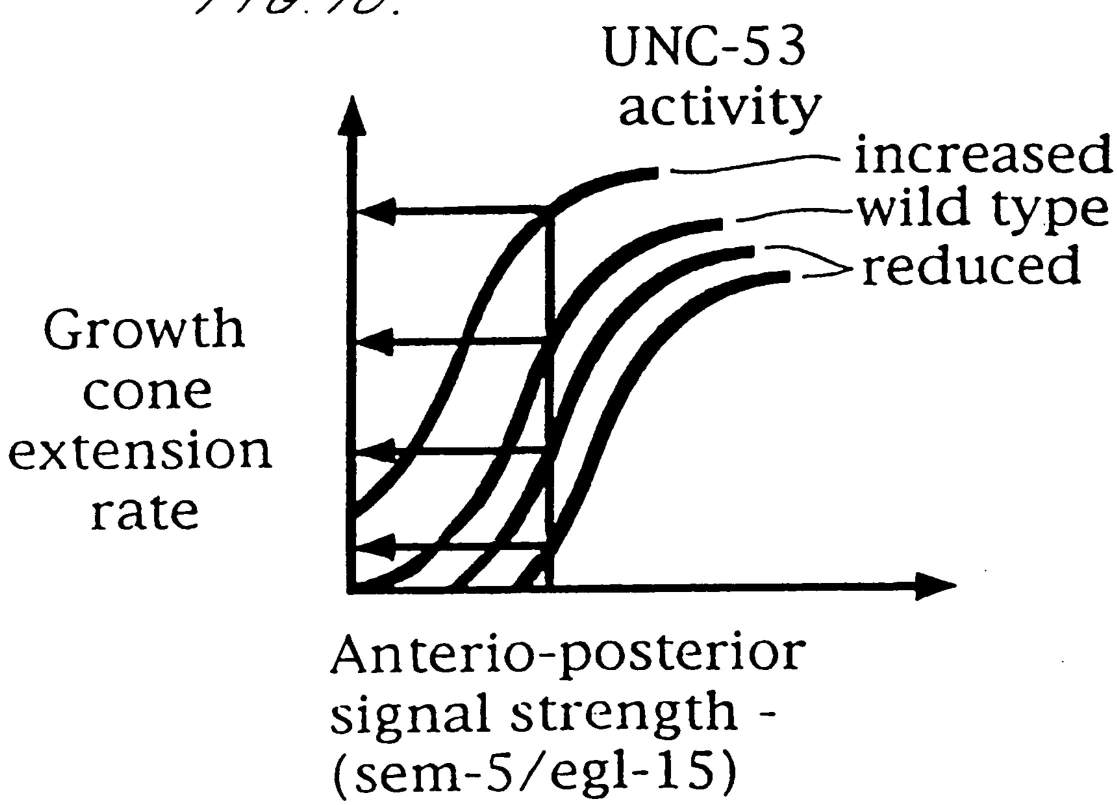
UNC-53
activity
increased
wild type
reduced
Growth
cone
extension
rate
Anterio-posterior
signal strength -
(sem-5/egl-15)

FIG. H.

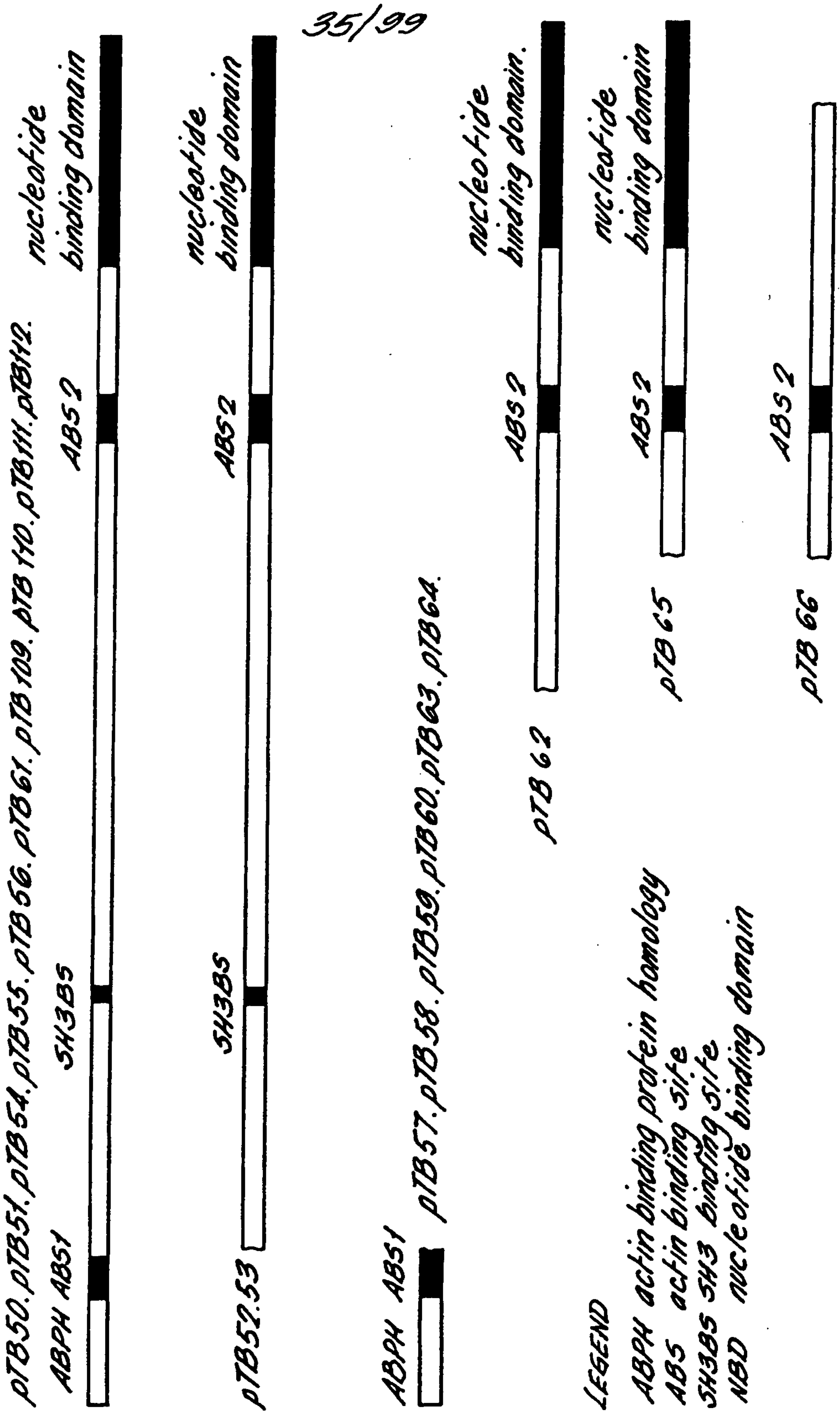

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FIG. 12.

5'ataagaatgcggccgccgccATGACGACGTCAAATGTAGAATTGATA (oligo BG03)

5'ggaattccaaccatATGACGACGTCAAATGTAGAATTGATA (oligo BG01)

ATGACGACGTCAAATGTAGAATTGATACCAATCTACACGGATTGGGCCAATCGGCACCTTTCG

AAGGGCAGCTTATCAAAGTCGATTAGGGATATTTCCAATGATTTTCGCGACTATCGACTGGTT

TCTCAGCTTATTAATGTGATCGTTCCGATCAACGAATTCTCGCCTGCATTCACGAAACGTTTG

GCAAAAATCACATCGAACCTGGATGGCCTCGAAACGTGTCTCGACTACCTGAAAAATCTGGGT

CTCGACTGCTCGAAACTCACCAAAACCGATATCGACAGCGGAAACTTGGGTGCAGTTCTCCAG

CTGCTCTTCCTGCTCTCCACCTACAAGCAGAAGCTTCGGCAACTGAAAAAAGATCAGAAGAAA

TTGGAGCAACTACCCACATCCATTATGCCACCCGCGGTTTCTAAATTACCCTCGCCACGTGTC

(oligo BG02)GTAGGTAATACGGTGGGCGCCAAActcctaggcgc-5'

FIG. 13.

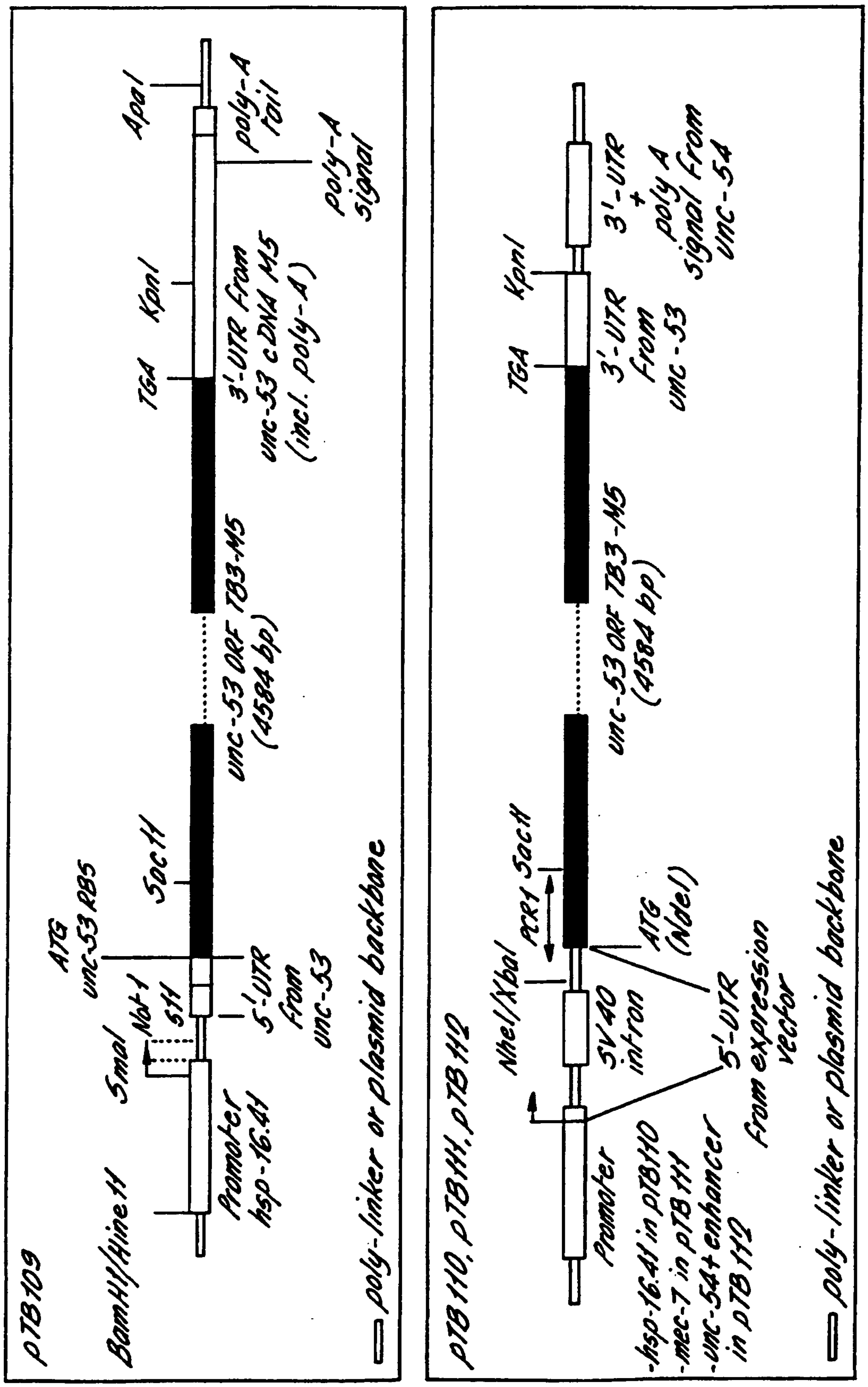
pTB109
BamHI/HincII
Promoter hsp-16.41
SmaI
Not-1
SH
5'-UTR from unc-53
ATG unc-53 RBS
SacII
unc-53 ORF TB3-M5 (4584 bp)
TGA
3'-UTR from unc-53 cDNA M5 (incl. poly-A)
KpnI
poly-A signal
poly-A tail
ApaI
poly-linker or plasmid backbone
pTB110, pTB111, pTB112
Promoter
-hsp-16.41 in pTB110
-mec-7 in pTB111
-unc-54+enhancer in pTB112
5'-UTR from expression vector
NheI/XbaI
SV40 intron
PCR1
SacII
ATG (NdeI)
unc-53 ORF TB3-M5 (4584 bp)
TGA
3'-UTR from unc-53
KpnI
3'-UTR + poly A signal from unc-54
poly-linker or plasmid backbone

FIG. 14a.

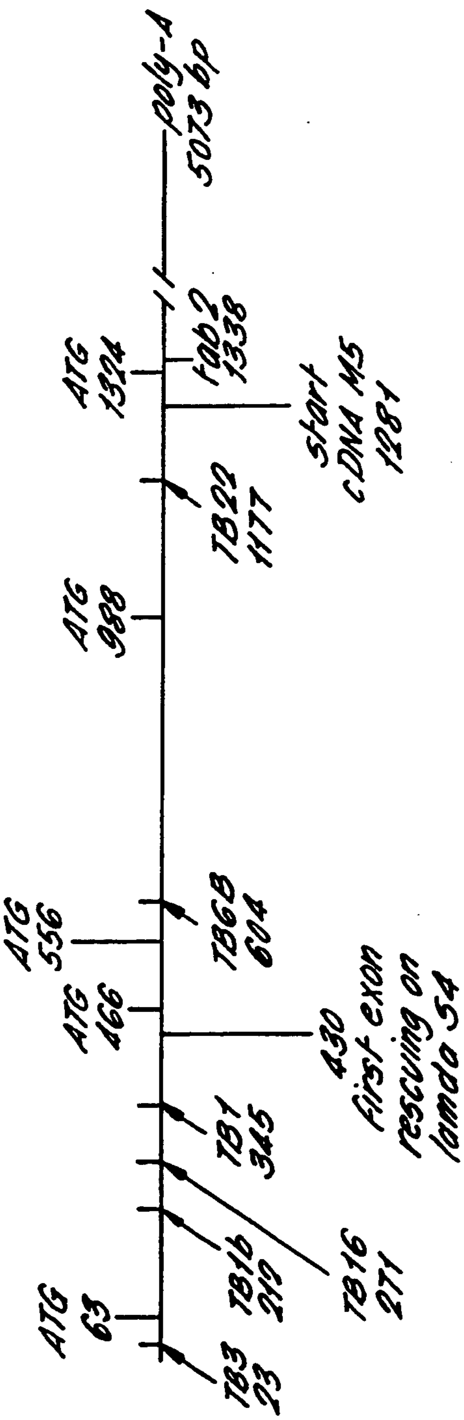
poly-A
5073 bp
tab2
1338
ATG
1324
start
cDNA M5
1281
TB22
1177
ATG
988
ATG
556
TB6B
604
ATG
466
430
first exon
rescuing on
lamda 54
TB1
345
TB16
271
TB1b
219
ATG
63
TB3
23

FIG. 14b. 39/99

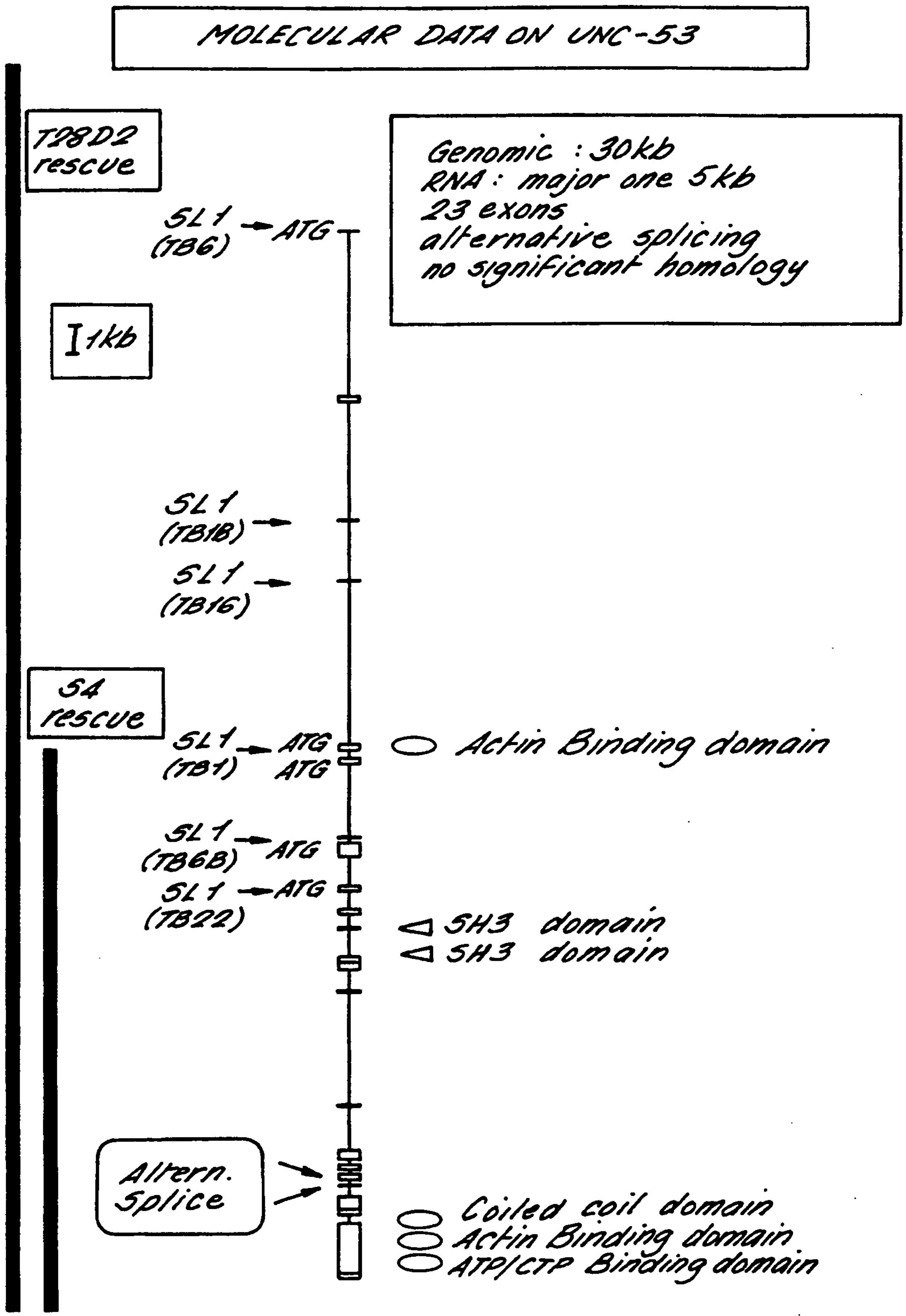
MOLECULAR DATA ON UNC-53
T28D2
rescue
Genomic : 30kb
RNA : major one 5kb
23 exons
alternative splicing
no significant homology
SL1 (TB6) ATG
1kb
SL1 (TB18)
SL1 (TB16)
S4
rescue
SL1 (TB1) ATG ATG
Actin Binding domain
SL1 (TB6B) ATG
SL1 (TB22) ATG
SH3 domain
SH3 domain
Altern.
Splice
Coiled coil domain
Actin Binding domain
ATP/GTP Binding domain

FIG. 14c.

S4

5'
gatcagaagaaattggagcaactacccacatccattatgccacccgcggtttctaagtgagt
ttaattttgagtttacgactacaaaaatgtgttcttta

....

ccgccttctgacttcgtgacgacagtctcgacacgtggggttgcaggtaggagtggatgagt
cgaaactgataagatagtcatttgagatc 3'

Co-ordinates in ACEDB.
5' begins at position 2260 in C09HIO.
3' finishes at 3287 in F45 E10.

Total 16818 bp.

FIG. 15.

```
(a) aact        1 MSEEPTPVSGNDKQLLNKAWEITQKKTFTAWCNSHLRK--LGSSIEQIDTDFTDGIKLAQ
                                             :* * * ** *  *  **  *  ** *    :*
(b) unc-53                      1 MTTSNVELIPIYTDWANRHLSKGSLSKSIRDISNDFRDYRLVSQ
                                             :* * * **:*  :*  * *:  *:*: * :
(c) spectrin          40 FERSRIKALADEREVVQKKTFTKWVNSHLAR--VSCRITDLYKDLRDGRMLIK

                  +  +           +     +                                +    +
(d) aact          LLEVISNDPVFKVNKTPKLRRIH-NIQNVGLCLKHIESHGVKLVGIGAEELVDKNLKMTL
                  *::**     *    * :* :*  *:: :  **  :   *:    :   ::   **   *
(e) unc-53        LINVIVPINEFSPAFTKRLAKITSNLDGLETCLDYLKNLGLDCSKLTKTDIDSGNLGAVL
                  *::*:    *  *  **   :*   *: ::  : :**:  :   ::   **  **   **
(f) spectrin      LLEVL-S-GEMLPKPTKGKMRIHC-LENVDKALQFLKEQRVHLENMGSHDIVDGNHRLVL

(g) aact          GMIWTIILRFAIQDISIEEL------SAKEALLLWCQRKTEGYDRVKV
                   ::: ::  :  : :
(h) unc-53        QLLF-LLSTYK-QKLRQLKKDQKKLEQLPTSIMPPAVSKLPSPRVATS
                   *:  ::  :  : :
(i) spectrin      GLIWTIILRFQIQDIVVQTQEGRETRSAKDALLQFLKEQRVHLENMGS
                   <++++++++++++++++++++++++++++>
                     actin binding region in unc-53 ?
```

FIG. 16.

```
LLFLLSTYKQKLRQLKKDQKKLEQLPTS unc-53 106 to 133
    :   |   :||||:  ||   |::
ETVNVNKLKTENKQLKKEVDKLTNGPAT unc-53 1093 to 1120
```

FIG. 17.

	side on helix	1 4 7
		XphPpxP
(a)	UNC-53	KKDPPPAVPPRDT
(b)	UNC-53	TTDVPPLPPLKS
(c)	mSOS	EVPVPPPVPPRR
(d)	mSOS	HLDSPPAIPPR
(e)	mSOS	HSIAGPPVPPR
(f)	SOS 1359	YRAVPPPLPPRRK
(g)	SOS 1377	GELSPPPIPPRLN
(h)	Dynamin	APAVPPARPGS
(i)	dynamin	PAVPPARP
(j)	PI3K p85	PPRPLPVAPGS
(k)	PI3K p85	PAPALPPKPPK
(l)	AFAP-110	PPDNGPPPLPTSS
(m)	AFAP-110	PPQMPLPEIPQQW
(n)	3BP-1	APTMPPPLPPVPP
(o)	3BP-2	FPAYPPPPVPVP

FIG. 18. 43/99

```
V      1          11         21         31         41         51
       MTTSNVELIP IYTDWANRHL SKGSLSKSIR DISNDFRDYR LVSQLINVIV PINEFSPAFT

       ---------- ---------- ---------- ---------- ---------- ----------
H      1          11         21         31         41         51
V      61         71         81         91         101        111
       KRLAKITSNL DGLETCLDYL KNLGLDCSKL TKTDIDSGNL GAVLQLLFLL STYKQKLRQL

       ---------- ---------- ---------- ---------- ---------- ----------
H      61         71         81         91         101        111
V      121        131        141        151        161        171
       KKDQKKLEQL PTSIMPPAVS KLPSPRVATS ATASATNPNS NFPQMSTSRL QTPQSRISKI

       ---------- ---------- ---------- ---------- ---------- ----------
H      121        131        141        151        161        171
V      181        191        201        211        221        231
       DSSKIGIKPK TSGLKPPSSS TTSSNNTNSF RPSSRSSGNN NVGSTISTSA KSLESSSTYS

       ---------- ---------- ---------- ---------- ---------- ----------
H      181        191        201        211        221        231
V      241        251        261        271        281        291
       SISNLNRPTS QLQKPSRPQT QLVRVATTTK IGSSKLAAPK AVSTPKLASV KTIGAKQEPD

       ---------- ---------- ---------- ---------- ---------- ----------
H      241        251        261        271        281        291
V      301        311        321        331        341        351
       NSGGGGGGML KLKLFSSKNP SSSSNSPQPT RKAAAVPQQQ TLSKIAAPVK SGLKPPTSKL
               ** ********** ********** ********** ********** **********
       --------ML KLKLFSSKNP SSSSNSPQPT RKAAAVPQQQ TLSKIAAPVK SGLKPPTSKL
H      301        311        321        331        341        351
V      361        371        381        391        401        411
       GSATSMSKLC TPKVSYRKTD APIISQQDSK RCSKSSEEES GYAGFNSTSP TSSSTEGSLS
       ********** ********** ********** ********** ********** **********
       GSATSMSKLC TPKVSYRKTD APIISQQDSK RCSKSSEEES GYAGFNSTSP TSSSTEGSLS
H      361        371        381        391        401        411
V      421        431        441        451        461        471
       MHSTSSKSST SDEKSPSSDD LTLNASIVTA IRQPIAATPV SPNIINKPVE EKPTLAVKGV
       ********** ********** ********** ********** ********** **********
       MHSTSSKSST SDEKSPSSDD LTLNASIVTA IRQPIAATPV SPNIINKPVE EKPTLAVKGV
H      421        431        441        451        461        471
V      481        491        501        511        521        531
       KSTAKKDPPP AVPPRDTQPT IGVVSPIMAH KKLTNDPVIS EKPEPEKLQS MSIDTTDVPP
       ********** ********** ********** ********** ********** **********
       KSTAKKDPPP AVPPRDTQPT IGVVSPIMAH KKLTNDPVIS EKPEPEKLQS MSIDTTDVPP
H      481        491        501        511        521        531
V      541        551        561        571        581        591
       LPPLKSVVPL KMTSIRQPPT YDVLLKQGKI TSPVKSFGYE QSSASEDSIV AHASAQVTPP
       ********** ********** ********** ********** ********** **********
       LPPLKSVVPL KMTSIRQPPT YDVLLKQGKI TSPVKSFGYE QSSASEDSIV AHASAQVTPP
H      541        551        561        571        581        591
V      601        611        621        631        641        651
       TKTSGNHSLE RRMGKNKTSE SSGYTSDAGV AMCAKMREKL KEYDDMTRRA QNGYPDNFED
       ********** ********** ********** ********** ********** **********
       TKTSGNHSLE RRMGKNKTSE SSGYTSDAGV AMCAKMREKL KEYDDMTRRA QNGYPDNFED
H      601        611        621        631        641        651
V      661        671        681        691        701        711
       SSSLSSGISD NNELDDISTD DLSGVDMATV ASKHSDYSHF VRHPTSSSSK PRVPSRSSTS
       ********** ********** ********** ********** ********** **********
       SSSLSSGISD NNELDDISTD DLSGVDMATV ASKHSDYSHF VRHPTSSSSK PRVPSRSSTS
H      661        671        681        691        701        711
V      721        731        741        751        761        771
       VDSRSRAEQE NVYKLLSQCR TSQRGAAATS TFGQHSLRSP GYSSYSPHLS VSADKDTMSM
       ********** ********** ********** ********** ********** **********
```

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FIG. 18 CONTINUED.

VDSRSRAEQE NVYKLLSQCR TSQRGAAATS TFGQHSLRSP GYSSYSPHLS VSADKDTMSM
H 721 731 741 751 761 771

V 781 791 801 811 821 831
HSQTSRRPSS QKPSYSGQFH SLDRKCHLQE FTSTEHRMAA LLSPRRVPNS MSKYDSSGSY
********** ********** ********** ********** ********** **********
HSQTSRRPSS QKPSYSGQFH SLDRKCHLQE FTSTEHRMAA LLSPRRVPNS MSKYDSSGSY
H 781 791 801 811 821 831
V 841 851 861 871 881 891
SARSRGGSST GIYGETFQLH RLSDEKSPAH SAKSEMGSQL SLASTTAYGS LNEKYEHAIR
********** ********** ********** ********** ********** **********
SARSRGGSST GIYGETFQLH RLSDEKSPAH SAKSEMGSQL SLASTTAYGS LNEKYEHAIR
H 841 851 861 871 881 891
V 901 911 921 931 941 951
DMARDLECYK NTVDSLTKKQ ENYGALFDLF EQKLRKLTQH IDRSNLKPEE AIRFRQDIAH
********** ********** ********** ********** ********** **********
DMARDLECYK NTVDSLTKKQ ENYGALFDLF EQKLRKLTQH IDRSNLKPEE AIRFRQDIAH
H 901 911 921 931 941 951
V 961 971 981 991 1001 1011
LRDISNHLAS NSAHANEGAG ELLRQPSLES VASHRSSMSS SSKSSKQEKI SLSSFGKNKK
********** ********** ********** ********** ********** **********
LRDISNHLAS NSAHANEGAG ELLRQPSLES VASHRSSMSS SSKSSKQEKI SLSSFGKNKK
H 961 971 981 991 1001 1011
V 1021 1031 1041 1051 1061 1071
SWIRSSLSKF TKKKNKNYDE AHMPSISGSQ GTLDNIDVIE LKQELKERDS ALYEVRLDNL
********** ********** ********** ********** ********** **********
SWIRSSLSKF TKKKNKNYDE AHMPSISGSQ GTLDNIDVIE LKQELKERDS ALYEVRLDNL
H 1021 1031 1041 1051 1061 1071
V 1081 1091 1101 1111 1121 1131
DRAREVDVLR ETVNKLKTEN KQLKKEVDKL TNGPATRASS RASIPVIYDD EHVYDAACSS
********** ********** ********** ********** ********** **********
DRAREVDVLR ETVNKLKTEN KQLKKEVDKL TNGPATRASS RASIPVIYDD EHVYDAACSS
H 1081 1091 1101 1111 1121 1131
V 1141 1151 1161 1171 1181 1191
TSASQSSKRS SGCNSIKVTV NVDIAGEISS IVNPDKEIIV GYLAMSTSQS CWKDIDVSIL
********** ********** ********** ********** *****?**** **********
TSASQSSKRS SGCNSIKVTV NVDIAGEISS IVNPDKEIIV GYLAMPTSQS CWKDIDVSIL
H 1141 1151 1161 1171 1181 1191
V 1201 1211 1221 1231 1241 1251
GLFEVYLSRI DVEHQLGIDA RDSILGYQIG ELRRVIGDST TMITSHPTDI LTSSTTIRMF
********** ********** ********** ********** ********** **********
GLFEVYLSRI DVEHQLGIDA RDSILGYQIG ELRRVIGDST TMITSHPTDI LTSSTTIRMF
H 1201 1211 1221 1231 1241 1251
V 1261 1271 1281 1291 1301 1311
MHGAAQSRVD SLVLDMLLPK QMILQLVKSI LTERRLVLAG ATGIGKSKLA KTLAAYVSIR
********** ********** ********** ********** ********** **********
MHGAAQSRVD SLVLDMLLPK QMILQLVKSI LTERRLVLAG ATGIGKSKLA KTLAAYVSIR
H 1261 1271 1281 1291 1301 1311
V 1321 1331 1341 1351 1361 1371
TNQSEDSIVN ISIPENNKEE LLQVERRLEK ILRSKESCIV ILDNIPKNRI AFVVSVFANV
********** ********** ********** ********** ********** **********
TNQSEDSIVN ISIPENNKEE LLQVERRLEK ILRSKESCIV ILDNIPKNRI AFVVSVFANV
H 1321 1331 1341 1351 1361 1371
V 1381 1391 1401 1411 1421 1431
PLQNNEGPFV VCTVNRYQIP ELQIHHNFKM SVMSNRLEGF ILRYLRRRAV EDEYRLTVQM
********** ********** ********** ********** ********** **********
PLQNNEGPFV VCTVNRYQIP ELQIHHNFKM SVMSNRLEGF ILRYLRRRAV EDEYRLTVQM
H 1381 1391 1401 1411 1421 1431
V 1441 1451 1461 1471 1481 1491
PSELFKIIDF FPIALCAVNN FIEKTNSVDV TVGPRACLNC PLTVDGSREW FIRLWNENFI
********** ********** ********** ********** ********** **********

FIG. 18 CONTINUED

```
     PSELFKIIDF FPIALQAVNN FIEKTNSVDV TVGPRACLNC PLTVDGSREW FIRLWNENFI
H  1441      1451       1461       1471       1481       1491
V  1501      1511       1521       1531       1541       1551
     PYLERVARDG KKNLRSLHFL RGSHRHRL-- ---------- ---------- ----------
     ************
     PYLERVARDG KKTFGRCTSF EDPTDIVSEK WPWFDGENPE NVLKRLQLQD LVPSPANSSR
H  1501      1511       1521       1531       1541       1551

V    ---------- ---------- ---

     QHFNPLESLI QLHATKHQTI DNI
H  1561      1571       1581
```

FIG. 19.

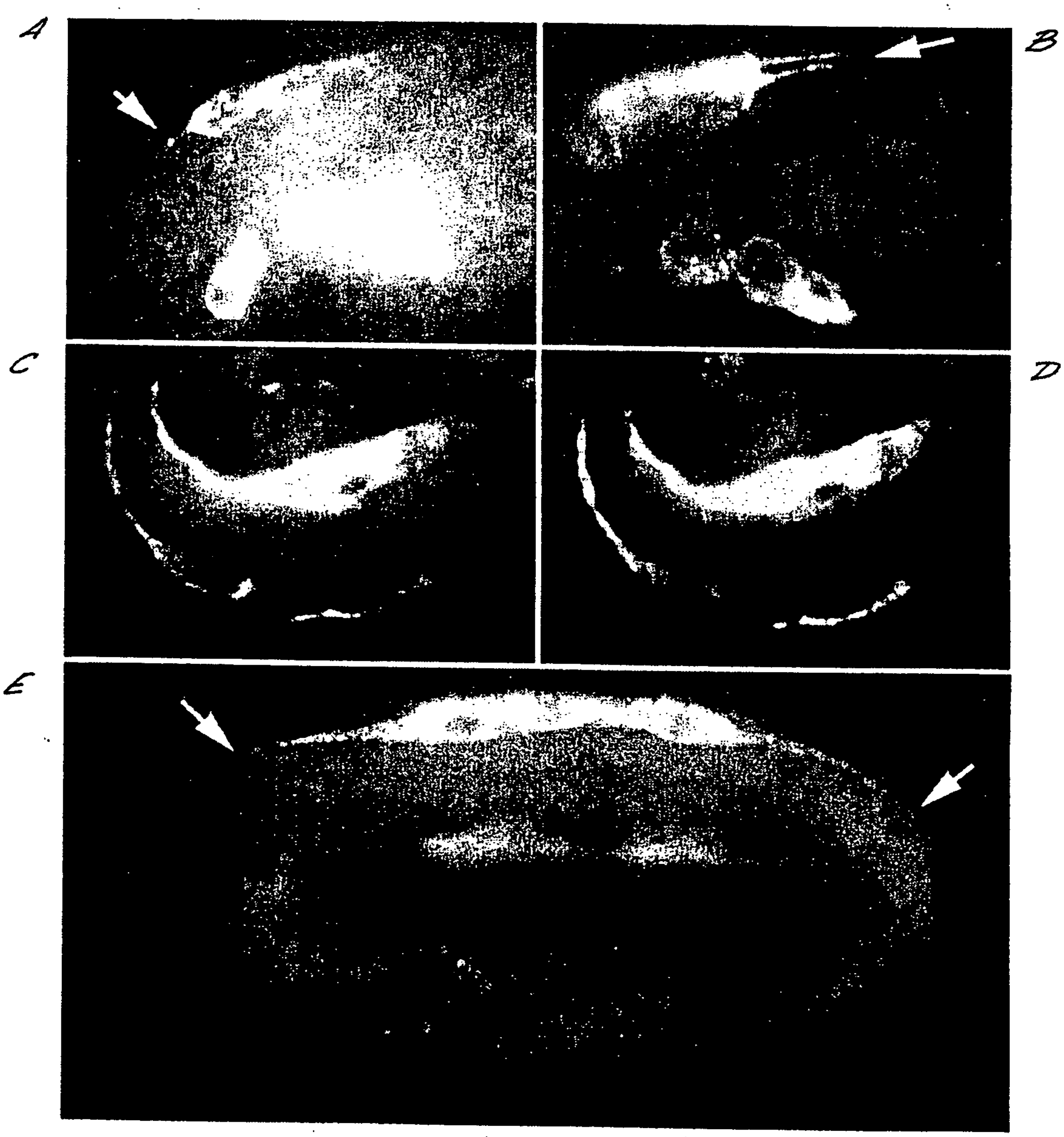


FIG. 20.

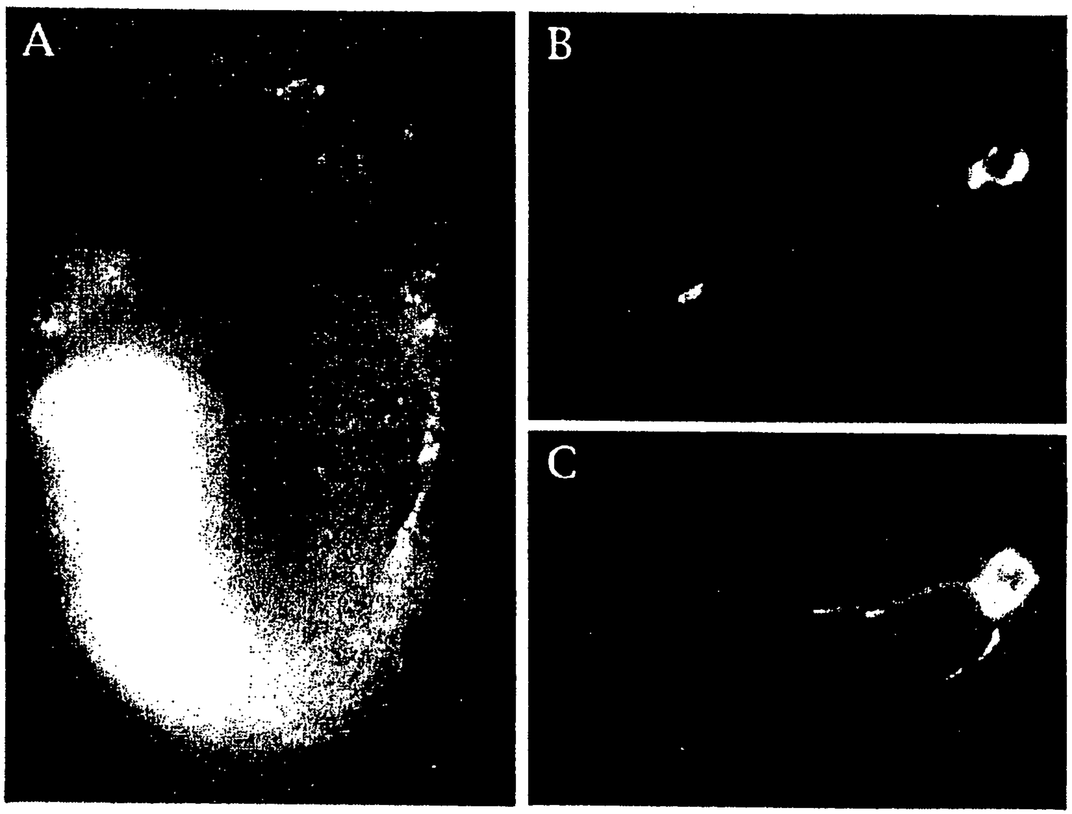


FIG. 21.

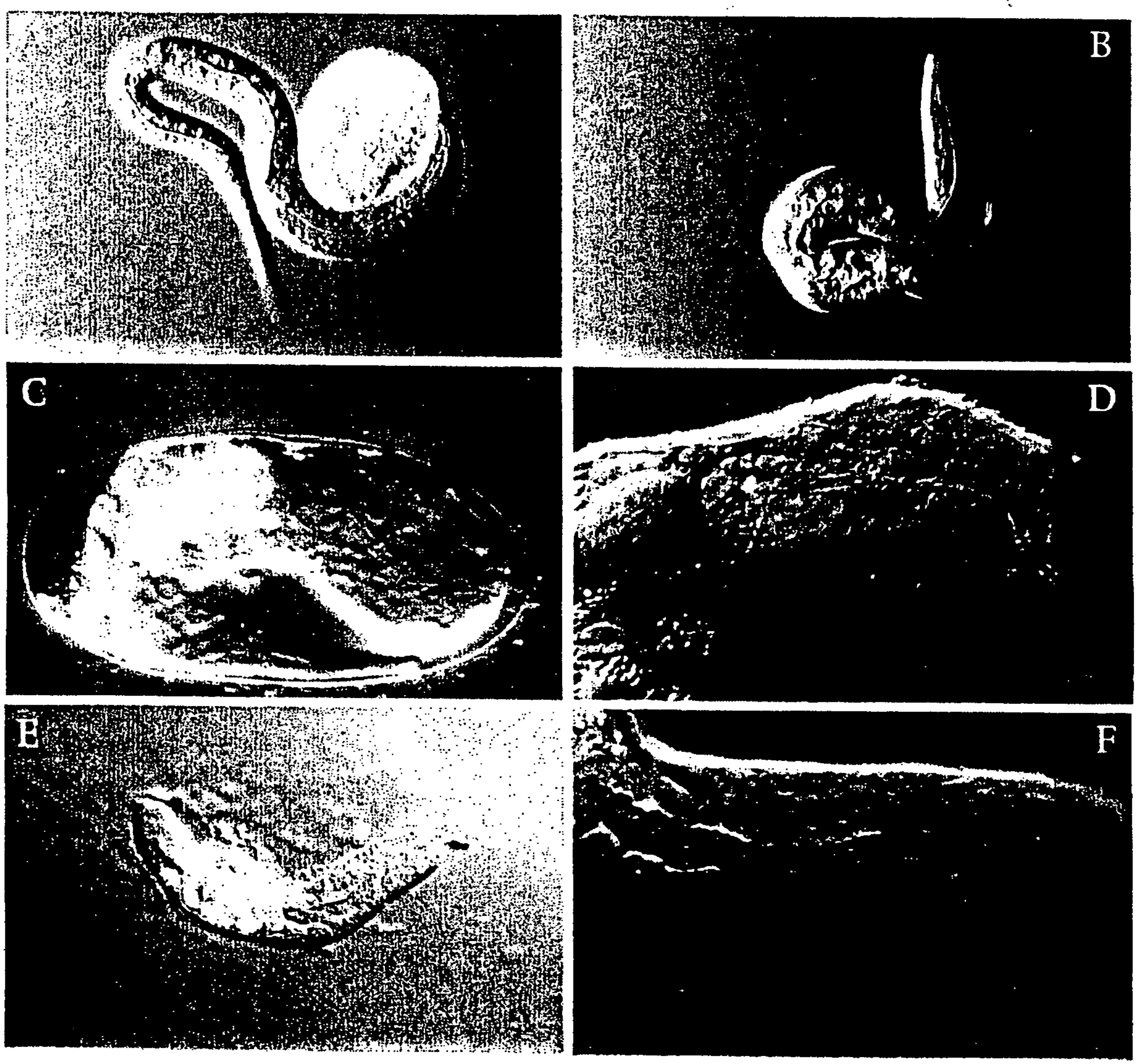
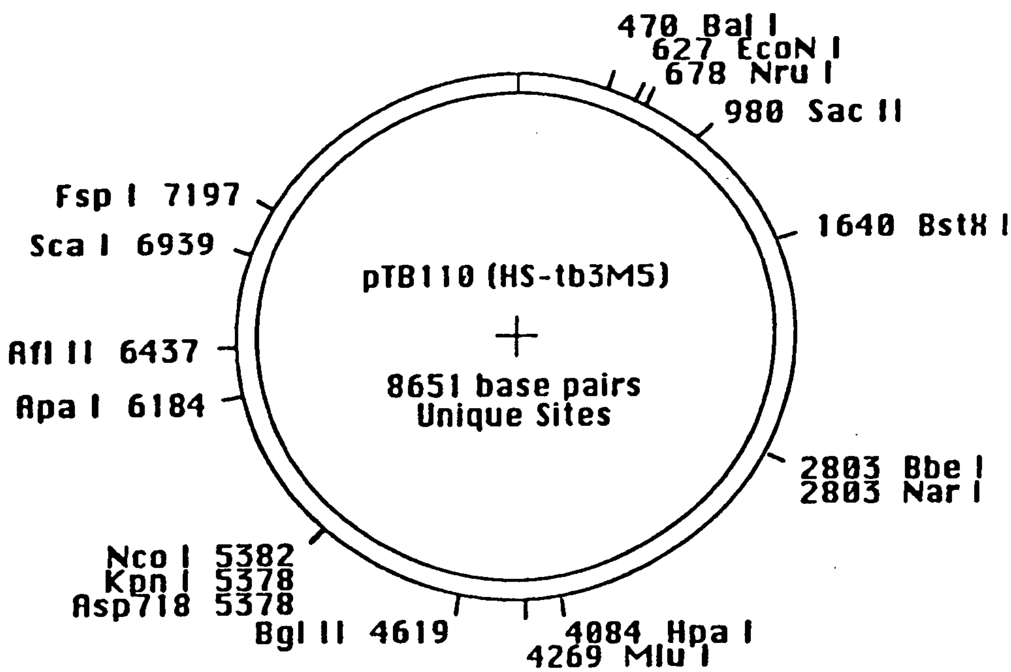


FIG. 22.



470 Bal I
627 EcoN I
678 Nru I
980 Sac II
1640 BstX I
2803 Bbe I
2803 Nar I
4084 Hpa I
4269 Mlu I
Bgl II 4619
Nco I 5382
Kpn I 5378
Asp718 5378
Apa I 6184
Afl II 6437
Sca I 6939
Fsp I 7197
pTB110 (HS-tb3M5)
8651 base pairs
Unique Sites

FIG. 23.

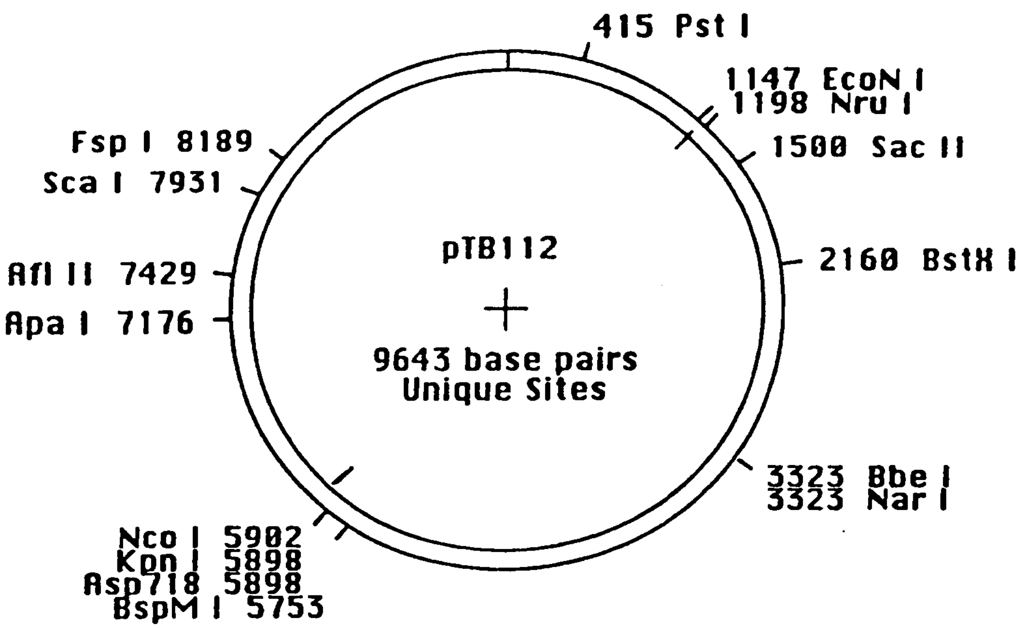

415 Pst I
1147 EcoN I
1198 Nru I
1500 Sac II
2160 BstX I
3323 Bbe I
3323 Nar I
Nco I 5902
Kpn I 5898
Asp718 5898
BspM I 5753
Apa I 7176
Afl II 7429
Sca I 7931
Fsp I 8189
pTB112
9643 base pairs
Unique Sites

FIG. 24.

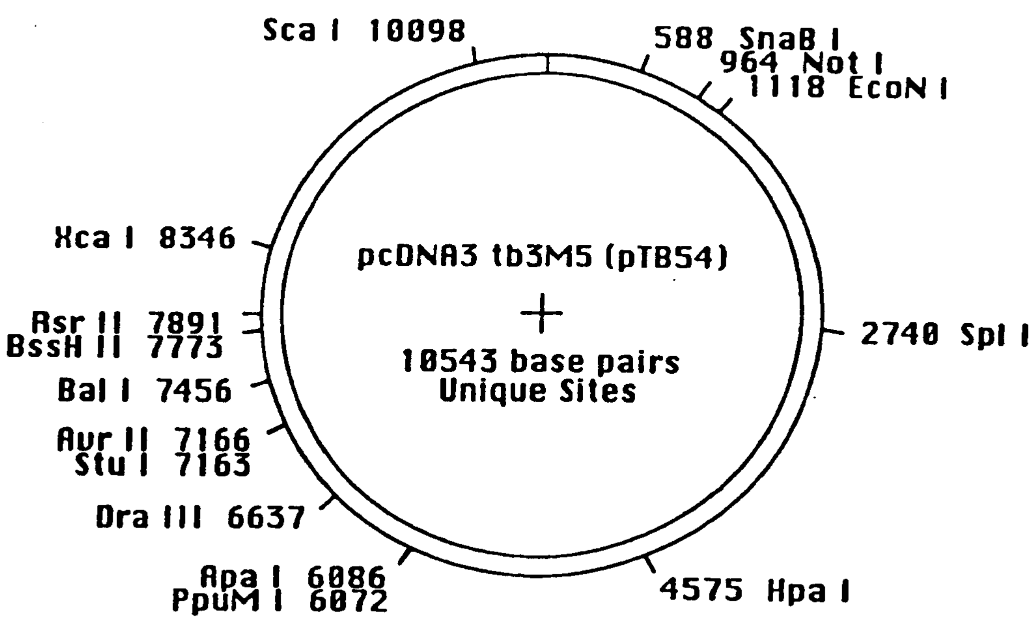

Sca I 10098
588 SnaB I
964 Not I
1118 EcoN I
Xca I 8346
pcDNA3 tb3M5 (pTB54)
Rsr II 7891
BssH II 7773
2740 Spl I
10543 base pairs
Unique Sites
Bal I 7456
Avr II 7166
Stu I 7163
Dra III 6637
Apa I 6086
PpuM I 6072
4575 Hpa I

FIG. 25.

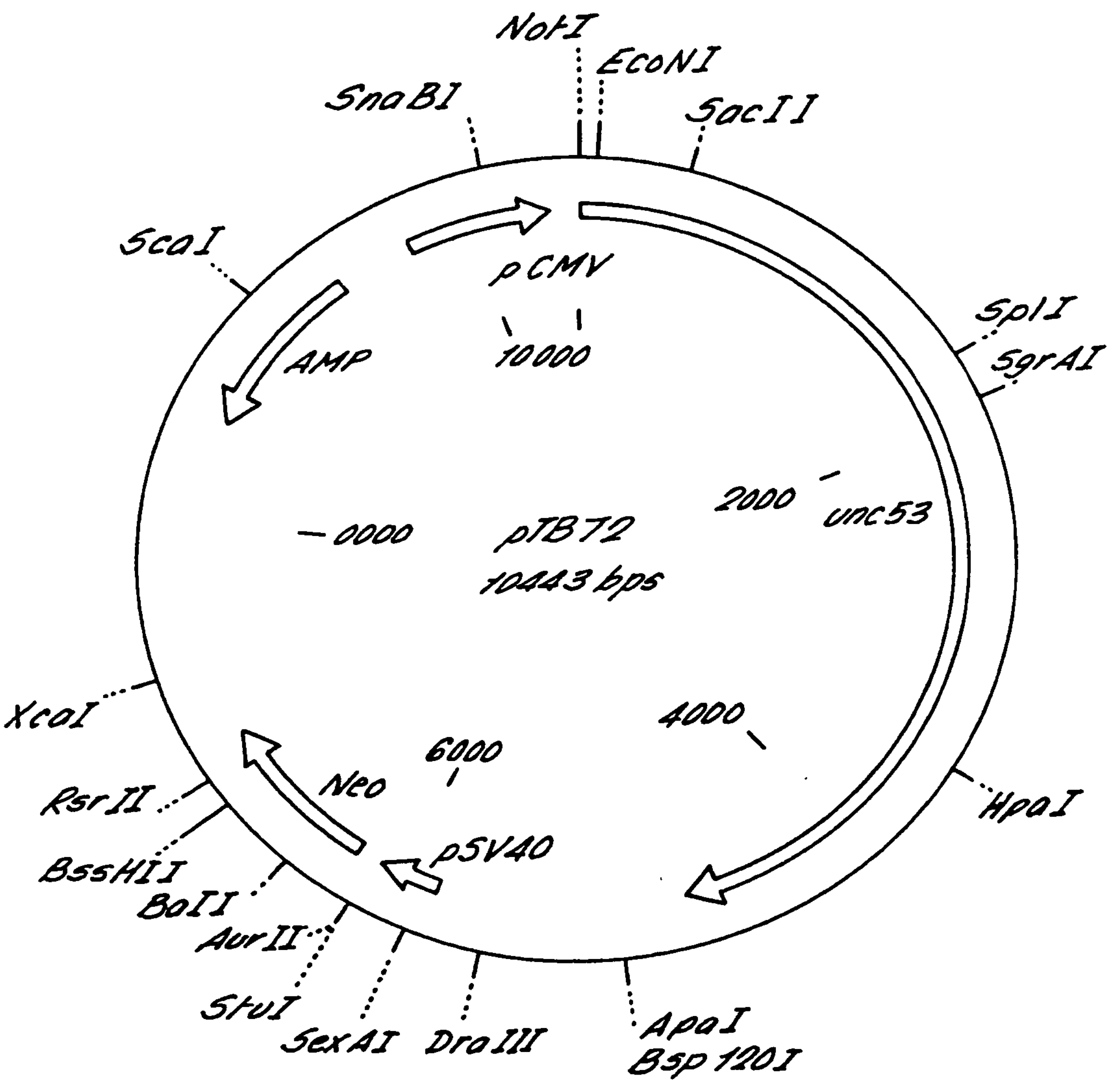

NotI
EcoNI
SnaBI
SacII
ScaI
pCMV
AMP
10000
SplI
SgrAI
2000
unc53
-0000
pTB72
10443 bps
XcaI
4000
6000
Neo
HpaI
RsrII
BssHII
pSV40
BoII
AurII
StuI
SexAI
DraIII
ApaI
Bsp120I

FIG. 26.

GGCCGCCGCC ATGACGACGT CAAATGTAGA ATTGATACCA ATCTACACGG ATTGGGCCAA 60
TCGGCACCTT TCGAAGGGCA GCTTATCAAA GTCGATTAGG GATATTTCCA ATGATTTTCG 120
CGACTATCGA CTGGTTTCTC AGCTTATTAA TGTGATCGTT CCGATCAACG AATTCTCGCC 180
TGCATTCACG AAACGTTTGG CAAAAATCAC ATCGAACCTG GATGGCCTCG AAACGTGTCT 240
CGACTACCTG AAAAATCTGG GTCTCGACTG CTCGAAACTC ACCAAAACCG ATATCGACAG 300
CGGAAACTTG GGTGCAGTTC TCCAGCTGCT CTTCCTGCTC TCCACCTACA AGCAGAAGCT 360
TCGGCAACTG AAAAAAGATC AGAAGAAATT GGAGCAACTA CCCACATCCA TTATGCCACC 420
CGCGGTTTCT AAATTACCCT CGCCACGTGT CGCCACGTCA GCAACCGCTT CAGCAACTAA 480
CCCAAATTCC AACTTTCCAC AAATGTCAAC ATCCAGGCTT CAGACTCCAC AGTCAAGAAT 540
ATCGAAAATT GATTCATCAA AGATTGGTAT CAAGCCAAAG ACGTCTGGAC TTAAACCACC 600
CTCATCATCA ACCACTTCAT CAAATAATAC AAATTCATTC CGTCCGTCGA GCCGTTCGAG 660
TGGCAATAAT AATGTTGGCT CGACGATATC CACATCTGCG AAGAGCTTAG AATCATCATC 720
AACGTACAGC TCTATTTCGA ATCTAAACCG ACCTACCTCC CAACTCCAAA AACCTTCTAG 780
ACCACAAACC CAGCTAGTTC GTGTTGCTAC AACTACAAAA ATCGGAAGCT CAAAGCTAGC 840
CGCTCCGAAA GCCGTGAGCA CCCCAAAACT TGCTTCTGTG AAGACTATTG GAGCAAAACA 900
AGAGCCCGAT AACAGCGGTG GTGGTGGTGG TGGAATGCTG AAATTAAAGT TATTCAGTAG 960
CAAAAACCCA TCTTCCTCAT CGAATAGCCC ACAACCTACG AGAAAGGCGG CGGCGGTGCC 1020
TCAACAACAA ACTTTGTCGA AAATCGCTGC CCCAGTGAAA AGTGGCCTGA AGCCGCCGAC 1080
CAGTAAGCTG GGAAGTGCCA CGTCTATGTC GAAGCTTTGT ACGCCAAAAG TTTCCTACCG 1140
TAAAACGGAC GCCCCAATCA TATCTCAACA AGACTCGAAA CGATGCTCAA AGAGCAGTGA 1200
AGAAGAGTCC GGATACGCTG GATTCAACAG CACGTCGCCA ACGTCATCAT CGACGGAAGG 1260
TTCCCTAAGC ATGCATTCCA CATCTTCCAA GAGTTCAACG TCAGACGAAA AGTCTCCGTC 1320
ATCAGACGAT CTTACTCTTA ACGCCTCCAT CGTGACAGCT ATCAGACAGC CGATAGCCGC 1380
AACACCGGTT TCTCCAAATA TTATCAACAA GCCTGTTGAG GAAAAACCAA CACTGGCAGT 1440

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FIG. 26 CONTINUED. 54/99

GAAAGGAGTG AAAAGCACAG CGAAAAAAGA TCCACCTCCA GCTGTTCCGC CACGTGACAC 1500
CCAGCCAACA ATCGGAGTTG TTAGTCCAAT TATGGCACAT AAGAAGTTGA CAAATGACCC 1560
CGTGATATCT GAAAAACCAG AACCTGAAAA GCTCCAATCA ATGAGCATCG ACACGACGGA 1620
CGTTCCACCG CTTCCACCTC TAAAATCAGT TGTTCCACTT AAAATGACTT CAATCCGACA 1680
ACCACCAACG TACGATGTTC TTCTAAAACA AGGAAAAATC ACATCGCCTG TCAAGTCGTT 1740
TGGATATGAG CAGTCGTCCG CGTCTGAAGA CTCCATTGTG GCTCATGCGT CGGCTCAGGT 1800
GACTCCGCCG ACAAAAACTT CTGGTAATCA TTCGCTGGAG AGAAGGATGG GAAAGAATAA 1860
GACATCAGAA TCCAGCGGCT ACACCTCTGA CGCCGGTGTT GCGATGTGCG CCAAAATGAG 1920
GGAGAAGCTG AAAGAATACG ATGACATGAC TCGTCGAGCA CAGAACGGCT ATCCTGACAA 1980
CTTCGAAGAC AGTTCCTCCT TGTCGTCTGG AATATCCGAT AACAACGAGC TCGACGACAT 2040
ATCCACGGAC GATTTGTCCG GAGTAGACAT GGCAACAGTC GCCTCCAAAC ATAGCGACTA 2100
TTCCCACTTT GTTCGCCATC CCACGTCTTC TTCCTCAAAG CCCCGAGTCC CCAGTCGGTC 2160
CTCCACATCA GTCGATTCTC GATCTCGAGC AGAACAGGAG AATGTGTACA AACTTCTGTC 2220
CCAGTGCCGA ACGAGCCAAC GTGGCGCCGC TGCCACCTCA ACCTTCGGAC AACATTCGCT 2280
AAGATCCCCG GGATACTCAT CCTATTCTCC ACACTTATCA GTGTCAGCTG ATAAGGACAC 2340
AATGTCTATG CACTCACAGA CTAGTCGACG ACCTTCTTCA CAAAAACCAA GCTATTCAGG 2400
CCAATTTCAT TCACTTGATC GTAAATGCCA CCTTCAAGAG TTCACATCCA CCGAGCACAG 2460
AATGGCGGCT CTCTTGAGCC CGAGACGGGT GCCGAACTCG ATGTCGAAAT ATGATTCTTC 2520
AGGATCCTAC TCGGCGCGTT CCCGAGGTGG AAGCTCTACT GGTATCTATG GAGAGACGTT 2580
CCAACTGCAC AGACTATCCG ATGAAAAATC CCCCGCACAT TCTGCCAAAA GTGAGATGGG 2640
ATCCCAACTA TCACTGGCTA GCACGACAGC ATATGGATCT CTCAATGAGA AGTACGAACA 2700
TGCTATTCGG GACATGGCAC GTGACTTGGA GTGTTACAAG AACACTGTCG ACTCACTAAC 2760
CAAGAAACAG GAGAACTATG GAGCATTGTT TGATCTTTTT GAGCAAAAGC TTAGAAAACT 2820
CACTCAACAC ATTGATCGAT CCAACTTGAA GCCTGAAGAG GCAATACGAT TCAGGCAGGA 2880
CATTGCTCAT TTGAGGGATA TTAGCAATCA TCTTGCATCC AACTCAGCTC ATGCTAACGA 2940
AGGCGCTGGT GAGCTTCTTC GTCAACCATC TCTGGAATCA GTTGCATCCC ATCGATCATC 3000
GATGTCATCG TCGTCGAAAA GCAGCAAGCA GGAGAAGATC AGCTTGAGCT CGTTTGGCAA 3060
GAACAAGAAG AGCTGGATCC GCTCCTCACT CTCCAAGTTC ACCAAGAAGA AGAACAAGAA 3120
CTACGACGAA GCACATATGC CATCAATTTC CGGATCTCAA GGAACTCTTG ACAACATTGA 3180
TGTGATTGAG TTGAAGCAAG AGCTCAAAGA ACGCGATAGT GCACTTTACG AAGTCCGCCT 3240
TGACAATCTG GATCGTGCCC GCGAAGTTGA TGTTCTGAGG GAGACAGTGA ACAAGTTGAA 3300
AACCGAGAAC AAGCAATTAA AGAAAGAAGT GGACAAACTC ACCAACGGTC CAGCCACTCG 3360

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FIG. 26 CONTINUED. 55/99

TGCTTCTTCC CGCGCCTCAA TTCCAGTTAT CTACGACGAT GAGCATGTCT ATGATGCAGC 3420
GTGTAGCAGT ACATCAGCTA GTCAATCTTC GAAACGATCC TCTGGCTGCA ACTCAATCAA 3480
GGTTACTGTA AACGTGGACA TCGCTGGAGA AATCAGTTCG ATCGTTAACC CGGACAAAGA 3540
GATAATCGTA GGATATCTTG CCATGTCAAC CAGTCAGTCA TGCTGGAAAG ACATTGATGT 3600
TTCTATTCTA GGACTATTTG AAGTCTACCT ATCCAGAATT GATGTGGAGC ATCAACTTGG 3660
AATCGATGCT CGTGATTCTA TCCTTGGCTA TCAAATTGGT GAACTTCGAC GCGTCATTGG 3720
AGACTCCACA ACCATGATAA CCAGCCATCC AACTGACATT CTTACTTCCT CAACTACAAT 3780
CCGAATGTTC ATGCACGGTG CCGCACAGAG TCGCGTAGAC AGTCTGGTCC TTGATATGCT 3840
TCTTCCAAAG CAAATGATTC TCCAACTCGT CAAGTCAATT TTGACAGAGA GACGTCTGGT 3900
GTTAGCTGGA GCAACTGGAA TTGGAAAGAG CAAACTGGCG AAGACCCTGG CTGCTTATGT 3960
ATCTATTCGA ACAAATCAAT CCGAAGATAG TATTGTTAAT ATCAGCATTC CTGAAAAACAA 4020
TAAAGAAGAA TTGCTTCAAG TGGAACGACG CCTGGAAAAG ATCTTGAGAA GCAAAGAATC 4080
ATGCATCGTA ATTCTAGATA ATATCCCAAA GAATCGAATT GCATTTGTTG TATCCGTTTT 4140
TGCAAATGTC CCACTTCAAA ACAACGAAGG TCCATTTGTA GTATGCACAG TCAACCGATA 4200
TCAAATCCCT GAGCTTCAAA TTCACCACAA TTTCAAAATG TCAGTAATGT CGAATCGTCT 4260
CGAAGGATTC ATCCTACGTT ACCTCCGACG ACGGGCGGTA GAGGATGAGT ATCGTCTAAC 4320
TGTACAGATG CCATCAGAGC TCTTCAAAAT CATTGACTTC TTCCCAATAG CTCTTCAGGC 4380
CGTCAATAAT TTTATTGAGA AAACGAATTC TGTTGATGTG ACAGTTGGTC CAAGAGCATG 4440
CTTGAACTGT CCTCTAACTG TCGATGGATC CCGTGAATGG TTCATTCGAT TGTGGAATGA 4500
GAACTTCATT CCATATTTGG AACGTGTTGC TAGAGATGGC AAAAAAACCT TCGGTCGCTG 4560
CACTTCCTTC GAGGATCCCA CCGACATCGT CTCTAAAAAA TGGCCGTGGT TCGATGGTGA 4620
AAACCCGGAG AATGTGCTCA AACGTCTTCA ACTCCAAGAC CTCGTCCCGT CACCTGCCAA 4680
CTCATCCCGA CAACACTTCA ATCCCCTCGA GTCGTTGATC CAATTGCATG CTACCAAGCA 4740
TCAGACCATC GACAACATTT GAACAGAAGA CTCTAATCTT CTCTCGCCTC TCCCCCGCTT 4800
TCCTTATCTT CGTACCGGTA CCTGATGATT CCCCATTTTC CCCCTTTTCC CCCCAATTTC 4860
CCAGAACCTC CTGTTCCCTT TGTTCCTAGT CCTCCCGGGT GCCGACGCCG AAGCGATTTA 4920
AAAACCTTTT TCTTTCCGAA ACATTTCCCA TTGCTCATTA ATAGTCAAAT TGAATAAACA 4980
GTGTATGTAC TTAAAAAAAA AAAAAAAAAA ACTCGAGGGG GGGCCCTATT CTATAGTGTC 5040
ACCTAAATGC TAGAGCTCGC TGATCAGCCT CGACTGTGCC TTCTAGTTGC CAGCCATCTG 5100
TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCCTTT 5160
CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCATTCT ATTCTGGGGG 5220
GTGGGGTGGG GCAGGACAGC AAGGGGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG 5280

FIG. 26 CONTINUED.

ATGCGGTGGG CTCTATGGCT TCTGAGGCGG AAAGAACCAG CTGGGGCTCT AGGGGGTATC 5340
CCCACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG CGCAGCGTGA 5400
CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC TTTCTTCCCT TCCTTTCTCG 5460
CCACGTTCGC CGGCTTTCCC CGTCAAGCTC TAAATCGGGG CATCCCTTTA GGGTTCCGAT 5520
TTAGTGCTTT ACGGCACCTC GACCCCAAAA AACTTGATTA GGGTGATGGT TCACGTAGTG 5580
GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA 5640
GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT TCTTTTGATT 5700
TATAAGGGAT TTTGGGGATT TCGGCCTATT GGTTAAAAAA TGAGCTGATT TAACAAAAAT 5760
TTAACGCGAA TTAATTCTGT GGAATGTGTG TCAGTTAGGG TGTGGAAAGT CCCCAGGCTC 5820
CCCAGGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC AGGTGTGGAA 5880
AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA 5940
CCATAGTCCC GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT 6000
CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC GCCTCTGCCT 6060
CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC 6120
TCCCGGGAGC TTGTATATCC ATTTTCGGAT CTGATCAAGA GACAGGATGA GGATCGTTTC 6180
GCATGATTGA ACAAGATGGA TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT 6240
TCGGCTATGA CTGGGCACAA CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT 6300
CAGCGCAGGG GCGCCCGGTT CTTTTTGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC 6360
TGCAGGACGA GGCAGCGCGG CTATCGTGGC TGGCCACGAC GGGCGTTCCT TGCGCAGCTG 6420
TGCTCGACGT TGTCACTGAA GCGGGAAGGG ACTGGCTGCT ATTGGGCGAA GTGCCGGGGC 6480
AGGATCTCCT GTCATCTCAC CTTGCTCCTG CCGAGAAAGT ATCCATCATG GCTGATGCAA 6540
TGCGGCGGCT GCATACGCTT GATCCGGCTA CCTGCCCATT CGACCACCAA GCGAAACATC 6600
GCATCGAGCG AGCACGTACT CGGATGGAAG CCGGTCTTGT CGATCAGGAT GATCTGGACG 6660
AAGAGCATCA GGGGCTCGCG CCAGCCGAAC TGTTCGCCAG GCTCAAGGCG CGCATGCCCG 6720
ACGGCGAGGA TCTCGTCGTG ACCCATGGCG ATGCCTGCTT GCCGAATATC ATGGTGGAAA 6780
ATGGCCGCTT TTCTGGATTC ATCGACTGTG GCCGGCTGGG TGTGGCGGAC CGCTATCAGG 6840
ACATAGCGTT GGCTACCCGT GATATTGCTG AAGAGCTTGG CGGCGAATGG GCTGACCGCT 6900
TCCTCGTGCT TTACGGTATC GCCGCTCCCG ATTCGCAGCG CATCGCCTTC TATCGCCTTC 6960
TTGACGAGTT CTTCTGAGCG GGACTCTGGG GTTCGAAATG ACCGACCAAG CGACGCCCAA 7020
CCTGCCATCA CGAGATTTCG ATTCCACCGC CGCCTTCTAT GAAAGGTTGG GCTTCGGAAT 7080
CGTTTTCCGG GACGCCGGCT GGATGATCCT CCAGCGCGGG GATCTCATGC TGGAGTTCTT 7140
CGCCCACCCC AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC 7200

FIG. 26 CONTINUED.

AAATTTCACA AATAAAGCAT TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAACTCAT 7260
CAATGTATCT TATCATGTCT GTATACCGTC GACCTCTAGC TAGAGCTTGG CGTAATCATG 7320
GTCATAGCTG TTTCCTGTGT GAAATTGTTA TCCGCTCACA ATTCCACACA ACATACGAGC 7380
CGGAAGCATA AAGTGTAAAG CCTGGGGTGC CTAATGAGTG AGCTAACTCA CATTAATTGC 7440
GTTGCGCTCA CTGCCCGCTT TCCAGTCGGG AAACCTGTCG TGCCAGCTGC ATTAATGAAT 7500
CGGCCAACGC GCGGGGAGAG GCGGTTTGCG TATTGGGCGC TCTTCCGCTT CCTCGCTCAC 7560
TGACTCGCTG CGCTCGGTCG TTCGGCTGCG GCGAGCGGTA TCAGCTCACT CAAAGGCGGT 7620
AATACGGTTA TCCACAGAAT CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA 7680
GCAAAAGGCC AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTTCCATA GGCTCCGCCC 7740
CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT 7800
ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG TTCCGACCCT 7860
GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC TTTCTCAATG 7920
CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA 7980
CGAACCCCCC GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA 8040
CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA TTAGCAGAGC 8100
GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG 8160
AAGGACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG 8220
TAGCTCTTGA TCCGGCAAAC AAACCACCGC TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA 8280
GCAGATTACG CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC 8340
TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT TATCAAAAAG 8400
GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT AAATCAATCT AAAGTATATA 8460
TGAGTAAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA TCTCAGCGAT 8520
CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC GTGTAGATAA CTACGATACG 8580
GGAGGGCTTA CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC GCTCACCGGC 8640
TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC 8700
AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC 8760
GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG TGTCACGCTC 8820
GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA TCAAGGCGAG TTACATGATC 8880
CCCCATGTTG TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT CCGATCGTTG TCAGAAGTAA 8940
GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC TTACTGTCAT 9000
GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA 9060
GTGTATGCGG CGACCGAGTT GCTCTTGCCC GGCGTCAATA CGGGATAATA CCGCGCCACA 9120

FIG. 26 CONTINUED

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TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG   9180
GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGCACCCA ACTGATCTTC   9240
AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC   9300
AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA   9360
TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA   9420
GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC CTGACGTCGA   9480
CGGATCGGGA GATCTCCCGA TCCCCTATGG TCGACTCTCA GTACAATCTG CTCTGATGCC   9540
GCATAGTTAA GCCAGTATCT GCTCCCTGCT TGTGTGTTGG AGGTCGCTGA GTAGTGCGCG   9600
AGCAAAATTT AAGCTACAAC AAGGCAAGGC TTGACCGACA ATTGCATGAA GAATCTGCTT   9660
AGGGTTAGGC GTTTTGCGCT GCTTCGCGAT GTACGGGCCA GATATACGCG TTGACATTGA   9720
TTATTGACTA GTTATTAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG CCCATATATG   9780
GAGTTCCGCG TTACATAACT TACGGTAAAT GGCCCGCCTG GCTGACCGCC CAACGACCCC   9840
CGCCCATTGA CGTCAATAAT GACGTATGTT CCCATAGTAA CGCCAATAGG GACTTTCCAT   9900
TGACGTCAAT GGGTGGACTA TTTACGGTAA ACTGCCCACT TGGCAGTACA TCAAGTGTAT   9960
CATATGCCAA GTACGCCCCC TATTGACGTC AATGACGGTA AATGGCCCGC CTGGCATTAT  10020
GCCCAGTACA TGACCTTATG GGACTTTCCT ACTTGGCAGT ACATCTACGT ATTAGTCATC  10080
GCTATTACCA TGGTGATGCG GTTTTGGCAG TACATCAATG GGCGTGGATA GCGGTTTGAC  10140
TCACGGGGAT TTCCAAGTCT CCACCCCATT GACGTCAATG GGAGTTTGTT TTGGCACCAA  10200
AATCAACGGG ACTTTCCAAA ATGTCGTAAC AACTCCGCCC CATTGACGCA AATGGGCGGT  10260
AGGCGTGTAC GGTGGGAGGT CTATATAAGC AGAGCTCTCT GGCTAACTAG AGAACCCACT  10320
GCTTACTGGC TTATCGAAAT TAATACGACT CACTATAGGG AGACCCAAGC TTGGTACCGA  10380
GCTCGGATCC ACTAGTAACG GCCGCCAGTG TGCTGGAATT CTGCAGATAT CCATCACACT  10440
GGC                                                                10443
```

FIG. 27.

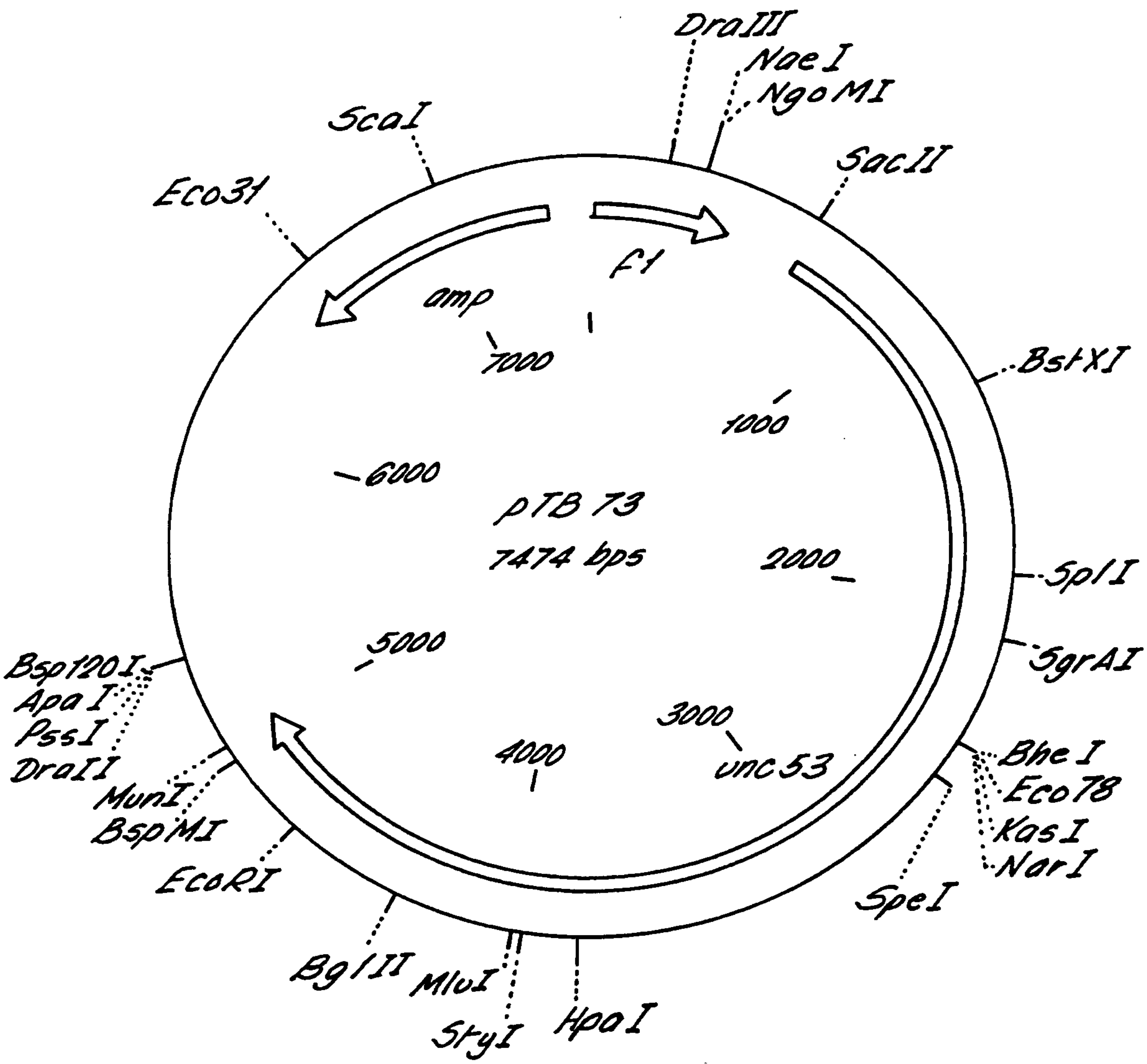
DraIII
NaeI
NgoMI
SacII
BstXI
SplI
SgrAI
BheI
Eco78
KasI
NarI
SpeI
HpaI
StyI
MluI
BglII
EcoRI
BspMI
MunI
DraII
PssI
ApaI
Bsp120I
Eco31
ScaI
amp
f1
unc53
pTB 73
7474 bps
1000
2000
3000
4000
5000
6000
7000

FIG. 28.

CTAAATTGTA AGCGTTAATA TTTTGTTAAA ATTCGCGTTA AATTTTTGTT AAATCAGCTC 60

ATTTTTTAAC CAATAGGCCG AAATCGGCAA AATCCCTTAT AAATCAAAAG AATAGACCGA 120

GATAGGGTTG AGTGTTGTTC CAGTTTGGAA CAAGAGTCCA CTATTAAAGA ACGTGGACTC 180

CAACGTCAAA GGGCGAAAAA CCGTCTATCA GGGCGATGGC CCACTACGTG AACCATCACC 240

CTAATCAAGT TTTTTGGGGT CGAGGTGCCG TAAAGCACTA AATCGGAACC CTAAAGGGAG 300

CCCCCGATTT AGAGCTTGAC GGGGAAAGCC GGCGAACGTG GCGAGAAAGG AAGGGAAGAA 360

AGCGAAAGGA GCGGGCGCTA GGGCGCTGGC AAGTGTAGCG GTCACGCTGC GCGTAACCAC 420

CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTCC CATTCGCCAT TCAGGCTGCG 480

CAACTGTTGG GAAGGGCGAT CGGTGCGGGC CTCTTCGCTA TTACGCCAGC TGGCGAAAGG 540

GGGATGTGCT GCAAGGCGAT TAAGTTGGGT AACGCCAGGG TTTTCCCAGT CACGACGTTG 600

TAAAACGACG GCCAGTGAGC GCGCGTAATA CGACTCACTA TAGGGCGAAT TGGAGCTCCA 660

CCGCGGTTTC TAAATTACCC TCGCCACGTG TCGCCACGTC AGCAACCGCT TCAGCAACTA 720

ACCCAAATTC CAACTTTCCA CAAATGTCAA CATCCAGGCT TCAGACTCCA CAGTCAAGAA 780

TATCGAAAAT TGATTCATCA AAGATTGGTA TCAAGCCAAA GACGTCTGGA CTTAAACCAC 840

CCTCATCATC AACCACTTCA TCAAATAATA CAAATTCATT CCGTCCGTCG AGCCGTTCGA 900

GTGGCAATAA TAATGTTGGC TCGACGATAT CCACATCTGC GAAGAGCTTA GAATCATCAT 960

CAACGTACAG CTCTATTTCG AATCTAAACC GACCTACCTC CCAACTCCAA AAACCTTCTA 1020

GACCACAAAC CCAGCTAGTT CGTGTTGCTA CAACTACAAA AATCGGAAGC TCAAAGCTAG 1080

CCGCTCCGAA AGCCGTGAGC ACCCCAAAAC TTGCTTCTGT GAAGACTATT GGAGCAAAAC 1140

AAGAGCCCGA TAACAGCGGT GGTGGTGGTG GTGGAATGCT GAAATTAAAG TTATTCAGTA 1200

GCAAAAACCC ATCTTCCTCA TCGAATAGCC CACAACCTAC GAGAAAGGCG GCGGCGGTGC 1260

CTCAACAACA AACTTTGTCG AAAATCGCTG CCCCAGTGAA AAGTGGCCTG AAGCCGCCGA 1320

CCAGTAAGCT GGGAAGTGCC ACGTCTATGT CGAAGCTTTG TACGCCAAAA GTTTCCTACC 1380

GTAAAACGGA CGCCCCAATC ATATCTCAAC AAGACTCGAA ACGATGCTCA AAGAGCAGTG 1440

AAGAAGAGTC CGGATACGCT GGATTCAACA GCACGTCGCC AACGTCATCA TCGACGGAAG 1500

GTTCCCTAAG CATGCATTCC ACATCTTCCA AGAGTTCAAC GTCAGACGAA AAGTCTCCGT 1560

CATCAGACGA TCTTACTCTT AACGCCTCCA TCGTGACAGC TATCAGACAG CCGATAGCCG 1620

CAACACCGGT TTCTCCAAAT ATTATCAACA AGCCTGTTGA GGAAAAACCA ACACTGGCAG 1680

TGAAAGGAGT GAAAAGCACA GCGAAAAAAG ATCCACCTCC AGCTGTTCCG CCACGTGACA 1740

CCCAGCCAAC AATCGGAGTT GTTAGTCCAA TTATGGCACA TAAGAAGTTG ACAAATGACC 1800

CCGTGATATC TGAAAAACCA GAACCTGAAA AGCTCCAATC AATGAGCATC GACACGACGG 1860

FIG. 28 CONTINUED. 61/99

ACGTTCCACC GCTTCCACCT CTAAAATCAG TTGTTCCACT TAAAATGACT TCAATCCGAC 1920
AACCACCAAC GTACGATGTT CTTCTAAAAC AAGGAAAAAT CACATCGCCT GTCAAGTCGT 1980
TTGGATATGA GCAGTCGTCC GCGTCTGAAG ACTCCATTGT GGCTCATGCG TCGGCTCAGG 2040
TGACTCCGCC GACAAAAACT TCTGGTAATC ATTCGCTGGA GAGAAGGATG GGAAAGAATA 2100
AGACATCAGA ATCCAGCGGC TACACCTCTG ACGCCGGTGT TGCGATGTGC GCCAAAATGA 2160
GGGAGAAGCT GAAAGAATAC GATGACATGA CTCGTCGAGC ACAGAACGGC TATCCTGACA 2220
ACTTCGAAGA CAGTTCCTCC TTGTCGTCTG GAATATCCGA TAACAACGAG CTCGACGACA 2280
TATCCACGGA CGATTTGTCC GGAGTAGACA TGGCAACAGT CGCCTCCAAA CATAGCGACT 2340
ATTCCCACTT TGTTCGCCAT CCCACGTCTT CTTCCTCAAA GCCCCGAGTC CCCAGTCGGT 2400
CCTCCACATC AGTCGATTCT CGATCTCGAG CAGAACAGGA GAATGTGTAC AAACTTCTGT 2460
CCCAGTGCCG AACGAGCCAA CGTGGCGCCG CTGCCACCTC AACCTTCGGA CAACATTCGC 2520
TAAGATCCCC GGGATACTCA TCCTATTCTC CACACTTATC AGTGTCAGCT GATAAGGACA 2580
CAATGTCTAT GCACTCACAG ACTAGTCGAC GACCTTCTTC ACAAAAACCA AGCTATTCAG 2640
GCCAATTTCA TTCACTTGAT CGTAAATGCC ACCTTCAAGA GTTCACATCC ACCGAGCACA 2700
GAATGGCGGC TCTCTTGAGC CCGAGACGGG TGCCGAACTC GATGTCGAAA TATGATTCTT 2760
CAGGATCCTA CTCGGCGCGT TCCCGAGGTG GAAGCTCTAC TGGTATCTAT GGAGAGACGT 2820
TCCAACTGCA CAGACTATCC GATGAAAAAT CCCCCGCACA TTCTGCCAAA AGTGAGATGG 2880
GATCCCAACT ATCACTGGCT AGCACGACAG CATATGGATC TCTCAATGAG AAGTACGAAC 2940
ATGCTATTCG GGACATGGCA CGTGACTTGG AGTGTTACAA GAACACTGTC GACTCACTAA 3000
CCAAGAAACA GGAGAACTAT GGAGCATTGT TTGATCTTTT TGAGCAAAAG CTTAGAAAAC 3060
TCACTCAACA CATTGATCGA TCCAACTTGA AGCCTGAAGA GGCAATACGA TTCAGGCAGG 3120
ACATTGCTCA TTTGAGGGAT ATTAGCAATC ATCTTGCATC CAACTCAGCT CATGCTAACG 3180
AAGGCGCTGG TGAGCTTCTT CGTCAACCAT CTCTGGAATC AGTTGCATCC CATCGATCAT 3240
CGATGTCATC GTCGTCGAAA AGCAGCAAGC AGGAGAAGAT CAGCTTGAGC TCGTTTGGCA 3300
AGAACAAGAA GAGCTGGATC CGCTCCTCAC TCTCCAAGTT CACCAAGAAG AAGAACAAGA 3360
ACTACGACGA AGCACATATG CCATCAATTT CCGGATCTCA AGGAACTCTT GACAACATTG 3420
ATGTGATTGA GTTGAAGCAA GAGCTCAAAG AACGCGATAG TGCACTTTAC GAAGTCCGCC 3480
TTGACAATCT GGATCGTGCC CGCGAAGTTG ATGTTCTGAG GGAGACAGTG AACAAGTTGA 3540
AAACCGAGAA CAAGCAATTA AAGAAAGAAG TGGACAAACT CACCAACGGT CCAGCCACTC 3600
GTGCTTCTTC CCGCGCCTCA ATTCCAGTTA TCTACGACGA TGAGCATGTC TATGATGCAG 3660
CGTGTAGCAG TACATCAGCT AGTCAATCTT CGAAACGATC CTCTGGCTGC AACTCAATCA 3720
AGGTTACTGT AAACGTGGAC ATCGCTGGAG AAATCAGTTC GATCGTTAAC CCGGACAAAG 3780

FIG. 28 CONTINUED. 62/99

AGATAATCGT AGGATATCTT GCCATGTCAA CCAGTCAGTC ATGCTGGAAA GACATTGATG 3840
TTTCTATTCT AGGACTATTT GAAGTCTACC TATCCAGAAT TGATGTGGAG CATCAACTTG 3900
GAATCGATGC TCGTGATTCT ATCCTTGGCT ATCAAATTGG TGAACTTCGA CGCGTCATTG 3960
GAGACTCCAC AACCATGATA ACCAGCCATC CAACTGACAT TCTTACTTCC TCAACTACAA 4020
TCCGAATGTT CATGCACGGT GCCGCACAGA GTCGCGTAGA CAGTCTGGTC CTTGATATGC 4080
TTCTTCCAAA GCAAATGATT CTCCAACTCG TCAAGTCAAT TTTGACAGAG AGACGTCTGG 4140
TGTTAGCTGG AGCAACTGGA ATTGGAAAGA GCAAACTGGC GAAGACCCTG GCTGCTTATG 4200
TATCTATTCG AACAAATCAA TCCGAAGATA GTATTGTTAA TATCAGCATT CCTGAAAACA 4260
ATAAAGAAGA ATTGCTTCAA GTGGAACGAC GCCTGGAAAA GATCTTGAGA AGCAAAGAAT 4320
CATGCATCGT AATTCTAGAT AATATCCCAA AGAATCGAAT TGCATTTGTT GTATCCGTTT 4380
TTGCAAATGT CCCACTTCAA AACAACGAAG GTCCATTTGT AGTATGCACA GTCAACCGAT 4440
ATCAAATCCC TGAGCTTCAA ATTCACCACA ATTTCAAAAT GTCAGTAATG TCGAATCGTC 4500
TCGAAGGATT CATCCTACGT TACCTCCGAC GACGGGCGGT AGAGGATGAG TATCGTCTAA 4560
CTGTACAGAT GCCATCAGAG CTCTTCAAAA TCATTGACTT CTTCCCAATA GCTCTTCAGG 4620
CCGTCAATAA TTTTATTGAG AAAACGAATT CTGTTGATGT GACAGTTGGT CCAAGAGCAT 4680
GCTTGAACTG TCCTCTAACT GTCGATGGAT CCCGTGAATG GTTCATTCGA TTGTGGAATG 4740
AGAACTTCAT TCCATATTTG GAACGTGTTG CTAGAGATGG CAAAAAAACC TTCGGTCGCT 4800
GCACTTCCTT CGAGGATCCC ACCGACATCG TCTCTAAAAA ATGGCCGTGG TTCGATGGTG 4860
AAAACCCGGA GAATGTGCTC AAACGTCTTC AACTCCAAGA CCTCGTCCCG TCACCTGCCA 4920
ACTCATCCCG ACAACACTTC AATCCCCTCG AGTCGTTGAT CCAATTGCAT GCTACCAAGC 4980
ATCAGACCAT CGACAACATT TGAACAGAAG ACTCTAATCT TCTCTCGCCT CTCCCCCGCT 5040
TTCCTTATCT TCGTACCGGT ACCTGATGAT TCCCCATTTT CCCCCTTTTC CCCCCAATTT 5100
CCCAGAACCT CCTGTTCCCT TTGTTCCTAG TCCTCCCGGG TGCCGACGCC GAAGCGATTT 5160
AAAAACCTTT TTCTTTCCGA AACATTTCCC ATTGCTCATT AATAGTCAAA TTGAATAAAC 5220
AGTGTATGTA CTTAAAAAAA AAAAAAAAAA AACTCGAGGG GGGGCCCGGT ACCCAGCTTT 5280
TGTTCCCTTT AGTGAGGGTT AATTGCGCGC TTGGCGTAAT CATGGTCATA GCTGTTTCCT 5340
GTGTGAAATT GTTATCCGCT CACAATTCCA CACAACATAC GAGCCGGAAG CATAAAGTGT 5400
AAAGCCTGGG GTGCCTAATG AGTGAGCTAA CTCACATTAA TTGCGTTGCG CTCACTGCCC 5460
GCTTTCCAGT CGGGAAACCT GTCGTGCCAG CTGCATTAAT GAATCGGCCA ACGCGCGGGG 5520
AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG 5580
GTCGTTCGGC TGCGGCGAGC GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA 5640
GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC 5700

FIG. 28 CONTINUED 63/99

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CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC 5760
AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG 5820
TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC 5880
CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT 5940
CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG 6000
CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC 6060
TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT 6120
GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT 6180
ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC 6240
AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA 6300
AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC 6360
GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC 6420
CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT 6480
GACAGTTACC AATGCTTAAT CAGTGAGGCA CCTATCTCAG CGATCTGTCT ATTTCGTTCA 6540
TCCATAGTTG CCTGACTCCC CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT 6600
GGCCCCAGTG CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA 6660
ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC CTGCAACTTT ATCCGCCTCC 6720
ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT TAATAGTTTG 6780
CGCAACGTTG TTGCCATTGC TACAGGCATC GTGGTGTCAC GCTCGTCGTT TGGTATGGCT 6840
TCATTCAGCT CCGGTTCCCA ACGATCAAGG CGAGTTACAT GATCCCCCAT GTTGTGCAAA 6900
AAAGCGGTTA GCTCCTTCGG TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA 6960
TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG TCATGCCATC CGTAAGATGC 7020
TTTTCTGTGA CTGGTGAGTA CTCAACCAAG TCATTCTGAG AATAGTGTAT GCGGCGACCG 7080
AGTTGCTCTT GCCCGGCGTC AATACGGGAT AATACCGCGC CACATAGCAG AACTTTAAAA 7140
GTGCTCATCA TTGGAAAACG TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG 7200
AGATCCAGTT CGATGTAACC CACTCGTGCA CCCAACTGAT CTTCAGCATC TTTTACTTTC 7260
ACCAGCGTTT CTGGGTGAGC AAAAACAGGA AGGCAAAATG CCGCAAAAAA GGGAATAAGG 7320
GCGACACGGA AATGTTGAAT ACTCATACTC TTCCTTTTTC AATATTATTG AAGCATTTAT 7380
CAGGGTTATT GTCTCATGAG CGGATACATA TTTGAATGTA TTTAGAAAAA TAAACAAATA 7440
GGGGTTCCGC GCACATTTCC CCGAAAAGTG CCAC 7474
```

FIG. 29.

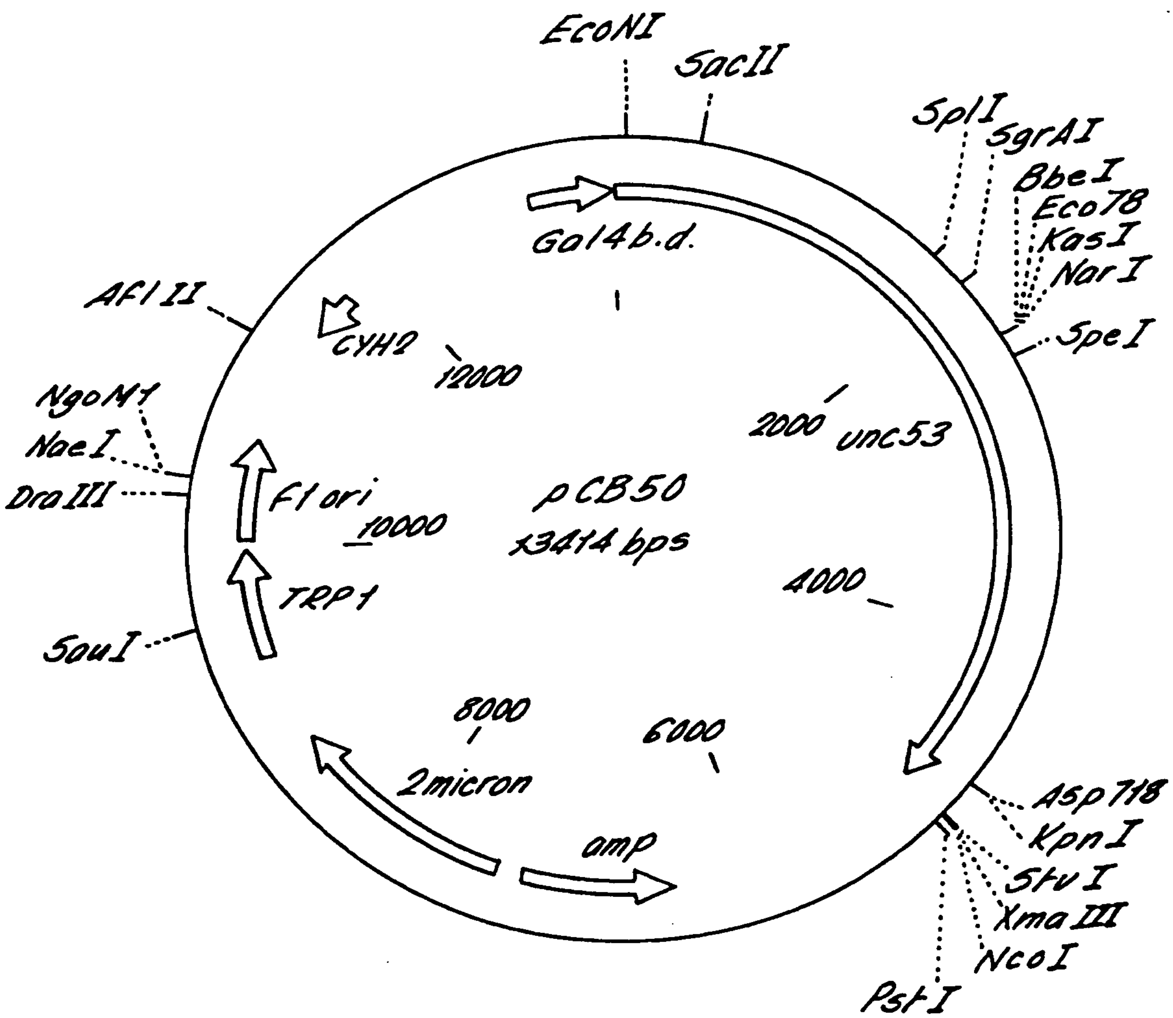
EcoNI
SacII
SplI
SgrAI
BbeI
Eco78
KasI
NarI
SpeI
AflII
NgoM1
NaeI
DraIII
SauI
Asp718
KpnI
StuI
XmaIII
NcoI
PstI
Gal4b.d.
CYH2
12000
2000
unc53
F1 ori
10000
pCB50
13414 bps
4000
TRP1
8000
6000
2micron
amp

FIG. 30.

```
TATGACGACG TCAAATGTAG AATTGATACC ATTCTACACG GATTGGGCCA ATCGGCACCT     60
TTCGAAGGGC AGCTTATCAA AGTCGATTAG GGATATTTCC AATGATTTTC GCGACTATCG    120
ACTGGTTTCT CAGCTTATTA ATGTGATCGT TCCGATCAAC GAATTCTCGC CTGCATTCAC    180
GAAACGTTTG GCAAAAATCA CATCGAACCT GGATGGCCTC GAAACGTGTC TCGACTACCT    240
GAAAAATCTG GGTCTCGACT GCTCGAAACT CACCAAAACC GATATCGACA GCGGAAACTT    300
GGGTGCAGTT CTCCAGCTGC TCTTCCTGCT CTCCACCTAC AAGCAGAAGC TTCGGCAACT    360
GAAAAAAGAT CAGAAGAAAT TGGAGCAACT ACCCACATCC ATTATGCCAC CCGCGGTTTC    420
TAAATTACCC TCGCCACGTG TCGCCACGTC AGCAACCGCT TCAGCAACTA ACCCAAATTC    480
CAACTTTCCA CAAATGTCAA CATCCAGGCT TCAGACTCCA CAGTCAAGAA TATCGAAAAT    540
TGATTCATCA AAGATTGGTA TCAAGCCAAA GACGTCTGGA CTTAAACCAC CCTCATCATC    600
AACCACTTCA TCAAATAATA CAAATTCATT CCGTCCGTCG AGCCGTTCGA GTGGCAATAA    660
TAATGTTGGC TCGACGATAT CCACATCTGC GAAGAGCTTA GAATCATCAT CAACGTACAG    720
CTCTATTTCG AATCTAAACC GACCTACCTC CCAACTCCAA AAACCTTCTA GACCACAAAC    780
CCAGCTAGTT CGTGTTGCTA CAACTACAAA AATCGGAAGC TCAAAGCTAG CCGCTCCGAA    840
AGCCGTGAGC ACCCCAAAAC TTGCTTCTGT GAAGACTATT GGAGCAAAAC AAGAGCCCGA    900
TAACAGCGGT GGTGGTGGTG GTGGAATGCT GAAATTAAAG TTATTCAGTA GCAAAAACCC    960
ATCTTCCTCA TCGAATAGCC CACAACCTAC GAGAAAGGCG GCGGCGGTGC CTCAACAACA   1020
AACTTTGTCG AAAATCGCTG CCCCAGTGAA AAGTGGCCTG AAGCCGCCGA CCAGTAAGCT   1080
GGGAAGTGCC ACGTCTATGT CGAAGCTTTG TACGCCAAAA GTTTCCTACC GTAAAACGGA   1140
CGCCCCAATC ATATCTCAAC AAGACTCGAA ACGATGCTCA AAGAGCAGTG AAGAAGAGTC   1200
CGGATACGCT GGATTCAACA GCACGTCGCC AACGTCATCA TCGACGGAAG GTTCCCTAAG   1260
```

FIG. 30 CONTINUED. 66/99

CATGCATTCC	ACATCTTCCA	AGAGTTCAAC	GTCAGACGAA	AAGTCTCCGT	CATCAGACGA	1320
TCTTACTCTT	AACGCCTCCA	TCGTGACAGC	TATCAGACAG	CCGATAGCCG	CAACACCGGT	1380
TTCTCCAAAT	ATTATCAACA	AGCCTGTTGA	GGAAAAACCA	ACACTGGCAG	TGAAAGGAGT	1440
GAAAAGCACA	GCGAAAAAAG	ATCCACCTCC	AGCTGTTCCG	CCACGTGACA	CCCAGCCAAC	1500
AATCGGAGTT	GTTAGTCCAA	TTATGGCACA	TAAGAAGTTG	ACAAATGACC	CCGTGATATC	1560
TGAAAAACCA	GAACCTGAAA	AGCTCCAATC	AATGAGCATC	GACACGACGG	ACGTTCCACC	1620
GCTTCCACCT	CTAAAATCAG	TTGTTCCACT	TAAAATGACT	TCAATCCGAC	AACCACCAAC	1680
GTACGATGTT	CTTCTAAAAC	AAGGAAAAAT	CACATCGCCT	GTCAAGTCGT	TTGGATATGA	1740
GCAGTCGTCC	GCGTCTGAAG	ACTCCATTGT	GGCTCATGCG	TCGGCTCAGG	TGACTCCGCC	1800
GACAAAAACT	TCTGGTAATC	ATTCGCTGGA	GAGAAGGATG	GGAAAGAATA	AGACATCAGA	1860
ATCCAGCGGC	TACACCTCTG	ACGCCGGTGT	TGCGATGTGC	GCCAAAATGA	GGGAGAAGCT	1920
GAAAGAATAC	GATGACATGA	CTCGTCGAGC	ACAGAACGGC	TATCCTGACA	ACTTCGAAGA	1980
CAGTTCCTCC	TTGTCGTCTG	GAATATCCGA	TAACAACGAG	CTCGACGACA	TATCCACGGA	2040
CGATTTGTCC	GGAGTAGACA	TGGCAACAGT	CGCCTCCAAA	CATAGCGACT	ATTCCCACTT	2100
TGTTCGCCAT	CCCACGTCTT	CTTCCTCAAA	GCCCCGAGTC	CCCAGTCGGT	CCTCCACATC	2160
AGTCGATTCT	CGATCTCGAG	CAGAACAGGA	GAATGTGTAC	AAACTTCTGT	CCCAGTGCCG	2220
AACGAGCCAA	CGTGGCGCCG	CTGCCACCTC	AACCTTCGGA	CAACATTCGC	TAAGATCCCC	2280
GGGATACTCA	TCCTATTCTC	CACACTTATC	AGTGTCAGCT	GATAAGGACA	CAATGTCTAT	2340
GCACTCACAG	ACTAGTCGAC	GACCTTCTTC	ACAAAAACCA	AGCTATTCAG	GCCAATTTCA	2400
TTCACTTGAT	CGTAAATGCC	ACCTTCAAGA	GTTCACATCC	ACCGAGCACA	GAATGGCGGC	2460
TCTCTTGAGC	CCGAGACGGG	TGCCGAACTC	GATGTCGAAA	TATGATTCTT	CAGGATCCTA	2520
CTCGGCGCGT	TCCCGAGGTG	GAAGCTCTAC	TGGTATCTAT	GGAGAGACGT	TCCAACTGCA	2580
CAGACTATCC	GATGAAAAAT	CCCCCGCACA	TTCTGCCAAA	AGTGAGATGG	GATCCCAACT	2640
ATCACTGGCT	AGCACGACAG	CATATGGATC	TCTCAATGAG	AAGTACGAAC	ATGCTATTCG	2700
GGACATGGCA	CGTGACTTGG	AGTGTTACAA	GAACACTGTC	GACTCACTAA	CCAAGAAACA	2760
GGAGAACTAT	GGAGCATTGT	TTGATCTTTT	TGAGCAAAAG	CTTAGAAAAC	TCACTCAACA	2820
CATTGATCGA	TCCAACTTGA	AGCCTGAAGA	GGCAATACGA	TTCAGGCAGG	ACATTGCTCA	2880
TTTGAGGGAT	ATTAGCAATC	ATCTTGCATC	CAACTCAGCT	CATGCTAACG	AAGGCGCTGG	2940
TGAGCTTCTT	CGTCAACCAT	CTCTGGAATC	AGTTGCATCC	CATCGATCAT	CGATGTCATC	3000
GTCGTCGAAA	AGCAGCAAGC	AGGAGAAGAT	CAGCTTGAGC	TCGTTTGGCA	AGAACAAGAA	3060
GAGCTGGATC	CGCTCCTCAC	TCTCCAAGTT	CACCAAGAAG	AAGAACAAGA	ACTACGACGA	3120
AGCACATATG	CCATCAATTT	CCGGATCTCA	AGGAACTCTT	GACAACATTG	ATGTGATTGA	3180

FIG. 30 CONTINUED. 67/99

GTTGAAGCAA GAGCTCAAAG AACGCGATAG TGCACTTTAC GAAGTCCGCC TTGACAATCT 3240
GGATCGTGCC CGCGAAGTTG ATGTTCTGAG GGAGACAGTG AACAAGTTGA AAACCGAGAA 3300
CAAGCAATTA AAGAAAGAAG TGGACAAACT CACCAACGGT CCAGCCACTC GTGCTTCTTC 3360
CCGCGCCTCA ATTCCAGTTA TCTACGACGA TGAGCATGTC TATGATGCAG CGTGTAGCAG 3420
TACATCAGCT AGTCAATCTT CGAAACGATC CTCTGGCTGC AACTCAATCA AGGTTACTGT 3480
AAACGTGGAC ATCGCTGGAG AAATCAGTTC GATCGTTAAC CCGGACAAAG AGATAATCGT 3540
AGGATATCTT GCCATGTCAA CCAGTCAGTC ATGCTGGAAA GACATTGATG TTTCTATTCT 3600
AGGACTATTT GAAGTCTACC TATCCAGAAT TGATGTGGAG CATCAACTTG GAATCGATGC 3660
TCGTGATTCT ATCCTTGGCT ATCAAATTGG TGAACTTCGA CGCGTCATTG GAGACTCCAC 3720
AACCATGATA ACCAGCCATC CAACTGACAT TCTTACTTCC TCAACTACAA TCCGAATGTT 3780
CATGCACGGT GCCGCACAGA GTCGCGTAGA CAGTCTGGTC CTTGATATGC TTCTTCCAAA 3840
GCAAATGATT CTCCAACTCG TCAAGTCAAT TTTGACAGAG AGACGTCTGG TGTTAGCTGG 3900
AGCAACTGGA ATTGGAAAGA GCAAACTGGC GAAGACCCTG GCTGCTTATG TATCTATTCG 3960
AACAAATCAA TCCGAAGATA GTATTGTTAA TATCAGCATT CCTGAAAACA ATAAAGAAGA 4020
ATTGCTTCAA GTGGAACGAC GCCTGGAAAA GATCTTGAGA AGCAAAGAAT CATGCATCGT 4080
AATTCTAGAT AATATCCCAA AGAATCGAAT TGCATTTGTT GTATCCGTTT TTGCAAATGT 4140
CCCACTTCAA AACAACGAAG GTCCATTTGT AGTATGCACA GTCAACCGAT ATCAAATCCC 4200
TGAGCTTCAA ATTCACCACA ATTTCAAAAT GTCAGTAATG TCGAATCGTC TCGAAGGATT 4260
CATCCTACGT TACCTCCGAC GACGGGCGGT AGAGGATGAG TATCGTCTAA CTGTACAGAT 4320
GCCATCAGAG CTCTTCAAAA TCATTGACTT CTTCCCAATA GCTCTTCAGG CCGTCAATAA 4380
TTTTATTGAG AAAACGAATT CTGTTGATGT GACAGTTGGT CCAAGAGCAT GCTTGAACTG 4440
TCCTCTAACT GTCGATGGAT CCCGTGAATG GTTCATTCGA TTGTGGAATG AGAACTTCAT 4500
TCCATATTTG GAACGTGTTG CTAGAGATGG CAAAAAAACC TTCGGTCGCT GCACTTCCTT 4560
CGAGGATCCC ACCGACATCG TCTCTAAAAA ATGGCCGTGG TTCGATGGTG AAAACCCGGA 4620
GAATGTGCTC AAACGTCTTC AACTCCAAGA CCTCGTCCCG TCACCTGCCA ACTCATCCCG 4680
ACAACACTTC AATCCCCTCG AGTCGTTGAT CCAATTGCAT GCTACCAAGC ATCAGACCAT 4740
CGACAACATT TGAACAGAAG ACTCTAATCT TCTCTCGCCT CTCCCCCGCT TTCCTTATCT 4800
TCGTACCGGT ACCTGATGAT TCCCCATTTT CCCCCTTTTC CCCCCAATTT CCCAGAACCT 4860
CCTGTTCCCT TTGTTCCTAG TCCTCCCGGG TGCCGACGCC GAAGCGATTT AAAAACCTTT 4920
TTCTTTCCGA AACATTTCCC ATTGCTCATT AATAGTCAAA TTGAATAAAC AGTGTATGTA 4980
CTTAAAAAAA AAAAAAAAAA AAAAAAAAAA GGCCTATGCG GCCGGGCCAT GGAGGCCGAA 5040
TTCCCGGGGA TCCGTCGACC TGCAGCCAAG CTAATTCCGG GCGAATTTCT TATGATTTAT 5100

FIG. 30 CONTINUED. 68/99

GATTTTTATT ATTAAATAAG TTATAAAAAA AATAAGTGTA TACAAATTTT AAAGTGACTC 5160
TTAGGTTTTA AAACGAAAAT TCTTGTTCTT GAGTAACTCT TTCCTGTAGG TCAGGTTGCT 5220
TTCTCAGGTA TAGCATGAGG TCGCTCTTAT TGACCACACC TCTACCGGCA TGCAAGCTTG 5280
GCGTAATCAT GGTCATAGCT GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC 5340
AACATACGAG CCGGAAGCAT AAAGTGTAAA GCCTGGGGTG CCTAATGAGT GAGGTAACTC 5400
ACATTAATTG CGTTGCGCTC ACTGCCCGCT TTCCAGTCGG GAAACCTGTC GTGCCAGCTG 5460
GATTAATGAA TCGGCCAACG CGCGGGGAGA GGCGGTTTGC GTATTGGGCG CTCTTCCGCT 5520
TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC GGCGAGCGGT ATCAGCTCAC 5580
TCAAAGGCGG TAATACGGTT ATCCACAGAA TCAGGGGATA ACGCAGGAAA GAACATGTGA 5640
GCAAAAGGCC AGCAAAAGGC CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTTCCAT 5700
AGGCTCCGCC CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC 5760
CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT 5820
GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG 5880
CTTTCTCATA GCTCACGCTG TAGGTATCTC AGTTCGGTGT AGGTCGTTCG CTCCAAGCTG 5940
GGCTGTGTGC ACGAACCCCC CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT 6000
CTTGAGTCCA ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG 6060
ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC 6120
GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA 6180
AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT 6240
GTTTGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT 6300
TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA 6360
TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC 6420
TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT 6480
ATCTCAGCGA TCTGTCTATT TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA 6540
ACTACGATAC GGGAGGGCTT ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA 6600
CGCTCACCGG CTCCAGATTT ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA 6660
AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA 6720
GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG 6780
GTGTCACGCT CGTCGTTTGG TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA 6840
GTTACATGAT CCCCCATGTT GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT 6900
GTCAGAAGTA AGTTGGCCGC AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT 6960
CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA 7020

FIG. 30 CONTINUED. 69/99

TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC CGGCGTCAAT ACGGGATAAT 7080
ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA 7140
AAACTCTCAA GGATCTTACC GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC 7200
AACTGATCTT CAGCATCTTT TACTTTCACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG 7260
CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC 7320
CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT 7380
GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA 7440
CCTGAACGAA GCATCTGTGC TTCATTTTGT AGAACAAAAA TGCAACGCGA GAGCGCTAAT 7500
TTTTCAAACA AAGAATCTGA GCTGCATTTT TACAGAACAG AAATGCAACG CGAAAGCGCT 7560
ATTTTACCAA CGAAGAATCT GTGCTTCATT TTTGTAAAAC AAAAATGCAA CGCGAGAGCG 7620
CTAATTTTTC AAACAAAGAA TCTGAGCTGC ATTTTTACAG AACAGAAATG CAACGCGAGA 7680
GCGCTATTTT ACCAACAAAG AATCTATACT TCTTTTTTGT TCTACAAAAA TGCATCCCGA 7740
GAGCGCTATT TTTCTAACAA AGCATCTTAG ATTACTTTTT TTCTCCTTTG TGCGCTCTAT 7800
AATGCAGTCT CTTGATAACT TTTTGCACTG TAGGTCCGTT AAGGTTAGAA GAAGGCTACT 7860
TTGGTGTCTA TTTTCTCTTC CATAAAAAAA GCCTGACTCC ACTTCCCGCG TTTACTGATT 7920
ACTAGCGAAG CTGCGGGTGC ATTTTTTCAA GATAAAGGCA TCCCCGATTA TATTCTATAC 7980
CGATGTGGAT TGCGCATACT TTGTGAACAG AAAGTGATAG CGTTGATGAT TCTTCATTGG 8040
TCAGAAAATT ATGAACGGTT TCTTCTATTT TGTCTCTATA TACTACGTAT AGGAAATGTT 8100
TACATTTTCG TATTGTTTTC GATTCACTCT ATGAATAGTT CTTACTACAA TTTTTTTGTC 8160
TAAAGAGTAA TACTAGAGAT AAACATAAAA AATGTAGAGG TCGAGTTTAG ATGCAAGTTC 8220
AAGGAGCGAA AGGTGGATGG GTAGGTTATA TAGGGATATA GCACAGAGAT ATATAGCAAA 8280
GAGATACTTT TGAGCAATGT TTGTGGAAGC GGTATTCGCA ATATTTTAGT AGCTCGTTAC 8340
AGTCCGGTGC GTTTTTGGTT TTTTGAAAGT GCGTCTTCAG AGCGCTTTTG GTTTTCAAAA 8400
GCGCTCTGAA GTTCCTATAC TTTCTAGAGA ATAGGAACTT CGGAATAGGA ACTTCAAAGC 8460
GTTTCCGAAA ACGAGCGCTT CCGAAAATGC AACGCGAGCT GCGCACATAC AGCTCACTGT 8520
TCACGTCGCA CCTATATCTG CGTGTTGCCT GTATATATAT ATACATGAGA AGAACGGCAT 8580
AGTGCGTGTT TATGCTTAAA TGCGTACTTA TATGCGTCTA TTTATGTAGG ATGAAAGGTA 8640
GTCTAGTACC TCCTGTGATA TTATCCCATT CCATGCGGGG TATCGTATGC TTCCTTCAGC 8700
ACTACCCTTT AGCTGTTCTA TATGCTGCCA CTCCTCAATT GGATTAGTCT CATCCTTCAA 8760
TGCTATCATT TCCTTTGATA TTGGATCATA TTAAGAAACC ATTATTATCA TGACATTAAC 8820
CTATAAAAAT AGGCGTATCA CGAGGCCCTT TCGTCTCGCG CGTTTCGGTG ATGACGGTGA 8880
AAACCTCTGA CACATGCAGC TCCCGGAGAC GGTCACAGCT TGTCTGTAAG CGGATGCCGG 8940

FIG. 30 CONTINUED 70/99

GAGCAGACAA GCCCGTCAGG GCGCGTCAGC GGGTGTTGGC GGGTGTCGGG GCTGGCTTAA 9000
CTATGCGGCA TCAGAGCAGA TTGTACTGAG AGTGCACCAT AGATCAACGA CATTACTATA 9060
TATATAATAT AGGAAGCATT TAATAGACAG CATCGTAATA TATGTGTACT TTGCAGTTAT 9120
GACGCCAGAT GGCAGTAGTG GAAGATATTC TTTATTGAAA AATAGCTTGT CACCTTACGT 9180
ACAATCTTGA TCCGGAGCTT TTCTTTTTTT GCCGATTAAG AATTAATTCG GTCGAAAAAA 9240
GAAAAGGAGA GGGCCAAGAG GGAGGGCATT GGTGACTATT GAGCACGTGA GTATACGTGA 9300
TTAAGCACAC AAAGGCAGCT TGGAGTATGT CTGTTATTAA TTTCACAGGT AGTTCTGGTC 9360
CATTGGTGAA AGTTTGCGGC TTGCAGAGCA CAGAGGCCGC AGAATGTGCT CTAGATTCCG 9420
ATGCTGACTT GCTGGGTATT ATATGTGTGC CCAATAGAAA GAGAACAATT GACCCGGTTA 9480
TTGCAAGGAA AATTTCAAGT CTTGTAAAAG CATATAAAAA TAGTTCAGGC ACTCCGAAAT 9540
ACTTGGTTGG CGTGTTTCGT AATCAACCTA AGGAGGATGT TTTGGCTCTG GTCAATGATT 9600
ACGGCATTGA TATCGTCCAA CTGCATGGAG ATGAGTCGTG GCAAGAATAC CAAGAGTTCC 9660
TCGGTTTGCC AGTTATTAAA AGACTCGTAT TTCCAAAAGA CTGCAACATA CTACTCAGTG 9720
CAGCTTCACA GAAACCTCAT TCGTTTATTC CCTTGTTTGA TTCAGAAGCA GGTGGGACAG 9780
GTGAACTTTT GGATTGGAAC TCGATTTCTG ACTGGGTTGG AAGGCAAGAG AGCCCCGAAA 9840
GCTTACATTT TATGTTAGCT GGTGGACTGA CGCCAGAAAA TGTTGGTGAT GCGCTTAGAT 9900
TAAATGGCGT TATTGGTGTT GATGTAAGCG GAGGTGTGGA GACAAATGGT GTAAAAGACT 9960
CTAACAAAAT AGCAAATTTC GTCAAAAATG CTAAGAAATA GGTTATTACT GAGTAGTATT 10020
TATTTAAGTA TTGTTTGTGC ACTTGCCGAT CTATGCGGTG TGAAATACCG CACAGATGCG 10080
TAAGGAGAAA ATACCGCATC AGGAAATTGT AAACGTTAAT ATTTTGTTAA AATTCGCGTT 10140
AAATTTTTGT TAAATCAGCT CATTTTTTAA CCAATAGGCC GAAATCGGCA AAATCCCTTA 10200
TAAATCAAAA GAATAGACCG AGATAGGGTT GAGTGTTGTT CCAGTTTGGA ACAAGAGTCC 10260
ACTATTAAAG AACGTGGACT CCAACGTCAA AGGGCGAAAA ACCGTCTATC AGGGCGATGG 10320
CCCACTACGT GAACCATCAC CCTAATCAAG TTTTTTGGGG TCGAGGTGCC GTAAAGCACT 10380
AAATCGGAAC CCTAAAGGGA GCCCCCGATT TAGAGCTTGA CGGGGAAAGC CGGCGAACGT 10440
GGCGAGAAAG GAAGGGAAGA AAGCGAAAGG AGCGGGCGCT AGGGCGCTGG CAAGTGTAGC 10500
GGTCACGCTG CGCGTAACCA CCACACCCGC CGCGCTTAAT GCGCCGCTAC AGGGCGCGTC 10560
GCGCCATTCG CCATTCAGGC TGCGCAACTG TTGGGAAGGG CGATCGGTGC GGGCCTCTTC 10620
GCTATTACGC CAGCTGGCGA AAGGGGGATG TGCTGCAAGG CGATTAAGTT GGGTAACGCC 10680
AGGGTTTTCC CAGTCACGAC GTTGTAAAAC GACGGCCAGT CGTCCAAGCT TTCGCGAGCT 10740
CGAGATCCCG AGCTTTGCAA ATTAAAGCCT TCGAGCGTCC CAAAACCTTC TCAAGCAAGG 10800
TTTTCAGTAT AATGTTACAT GCGTACACGC GTCTGTACAG AAAAAAAAGA AAAATTTGAA 10860

FIG. 30 CONTINUED. 71/99

ATATAAATAA CGTTCTTAAT ACTAACATAA CTATAAAAAA ATAAATAGGG ACCTAGACTT 10920
CAGGTTGTCT AACTCCTTCC TTTTCGGTTA GAGCGGATGT GGGGGGAGGG CGTGAATGTA 10980
AGCGTGACAT AACTAATTAC ATGATATCGA CAAAGGAAAA GGGGCCTGTT TACTCACAGG 11040
CTTTTTTCAA GTAGGTAATT AAGTCGTTTC TGTCTTTTTC CTTCTTCAAC CCACCAAAGG 11100
CCATCTTGGT ACTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT 11160
TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTCATA GAAATAATAC 11220
AGAAGTAGAT GTTGAATTAG ATTAAACTGA AGATATATAA TTTATTGGAA AATACATAGA 11280
GCTTTTTGTT GATGCGCTTA AGCGATCAAT TCAACAACAC CACCAGCAGC TCTGATTTTT 11340
TCTTCAGCCA ACTTGGAGAC GAATCTAGCT TTGACGATAA CTGGAACATT TGGGATTCTA 11400
CCCTTACCCA AGATCTTACC GTAACCGGCT GCCAAAGTGT CAATAACTGG AGCAGTTTCC 11460
TTAGAAGCAG ATTTCAAGTA TTGGTCTCTC TTGTCTTCTG GGATCAATGT CCACAATTTG 11520
TCCAAGTTCA AGACTGGCTT CCAGAAATGA GCTTGTTGCT TGTGGAAGTA TCTCATACCA 11580
ANCCTTACCG AAATAACCTG GATGGTATTT ATCCATGTTA ATTCTGTGGT GATGTTGACC 11640
ACCGGCCATA CCTCTACCAC CGGGGTGCTT TCTGTGCTTA CCGATACGAC CTTTACCGGC 11700
TGAGACGTGA CCTCTGTGCT TTCTAGTCTT AGTGAATCTG GAAGGCATTC TTGATTAGTT 11760
GGATGATTGT TCTGGGATTT AATGCAAAAA AATCACTAAG AAGGAAAAAA ATCAACGGAG 11820
AAAGCAAACG CCATCTTAAA TATACGGGAT ACAGATGAAA GGTTTGAACC TATCTGGGAA 11880
AATACGCATT AAACAAGCGA AAAACTGCGA GGAAAATTGT TTGCGTCTCT GCGGGCTATT 11940
CACGCGCCAG AGGAAAATAG GAAAAATAAC AGGGCATTAG AAAAATAATT TTGATTTTGG 12000
TAATGTGTGG GTCCCTGGTG TACAGATGTT ACATTGGTTA CAGTACTCTT GTTTTTGCTG 12060
TGTTTTTCGA TGAATCTCCA AAATGGTTGT TAGCACATGG AAGAGTCACC GATGCTAAGT 12120
TATCTCTATG TAAGCTACGT GGCGTGACTT TTGATGAAGC CGCACAAGAG ATACAGGATT 12180
GGCAACTGCA AATAGAATCT GGGGATCTAG ATATCCTTTT GTTGTTTCCG GGTGTACAAT 12240
ATGGACTTCC TCTTTTCTGG CAACCAAACC CATACATCGG GATTCCTATA ATACCTTCGT 12300
TGGTCTCCCT AACATGTAGG TGGCGGAGGG GAGATATACA ATAGAACAGA TACCAGACAA 12360
GACATAATGG GCTAAACAAG ACTACACCAA TTACACTGCC TCATTGATGG TGGTACATAA 12420
CGAACTAATA CTGTAGCCCT AGACTTGATA GCCATCATCA TATCGAAGTT TCACTACCCT 12480
TTTTCCATTT GCCATCTATT GAAGTAATAA TAGGCGCATG CAACTTCTTT TCTTTTTTTT 12540
TCTTTTCTCT CTCCCCCGTT GTTGTCTCAC CATATCCGCA ATGACAAAAA AAATGATGGA 12600
AGACACTAAA GGAAAAAATT AACGACAAAG ACAGCACCAA CAGATGTCGT TGTTCCAGAG 12660
CTGATGAGGG GTATCTTCGA ACACACGAAA CTTTTTCCTT CCTTCATTCA CGCACACTAC 12720
TCTCTAATGA GCAACGGTAT ACGGCCTTCC TTCCAGTTAC TTGAATTTGA AATAAAAAAA 12780

FIG. 30 CONTINUED.

```
GTTTGCCGCT TTGCTATCAA GTATAAATAG ACCTGCAATT ATTAATCTTT TGTTTCCTCG  12840
TCATTGTTCT CGTTCCCTTT CTTCCTTGTT TCTTTTTCTG CACAATATTT CAAGCTATAC  12900
CAAGCATACA ATCAACTCCA AGCTTGAAGC AAGCCTCCTG AAAGATGAAG CTACTGTCTT  12960
CTATCGAACA AGCATGCGAT ATTTGCCGAC TTAAAAAGCT CAAGTGCTCC AAAGAAAAAC  13020
CGAAGTGCGC CAAGTGTCTG AAGAACAACT GGGAGTGTCG CTACTCTCCC AAAACCAAAA  13080
GGTCTCCGCT GACTAGGGCA CATCTGACAG AAGTGGAATC AAGGCTAGAA AGACTGGAAC  13140
AGCTATTTCT ACTGATTTTT CCTCGAGAAG ACCTTGACAT GATTTTGAAA ATGGATTCTT  13200
TACAGGATAT AAAAGCATTG TTAACAGGAT TATTTGTACA AGATAATGTG AATAAAGATG  13260
CCGTCACAGA TAGATTGGCT TCAGTGGAGA CTGATATGCC TCTAACATTG AGACAGCATA  13320
GAATAAGTGC GACATCATCA TCGGAAGAGA GTAGTAACAA AGGTCAAAGA CAGTTGACTG  13380
TATCGCCGGA ATTGCAATAC CCAGCTTTGA CTCA                              13414
```

FIG. 31.

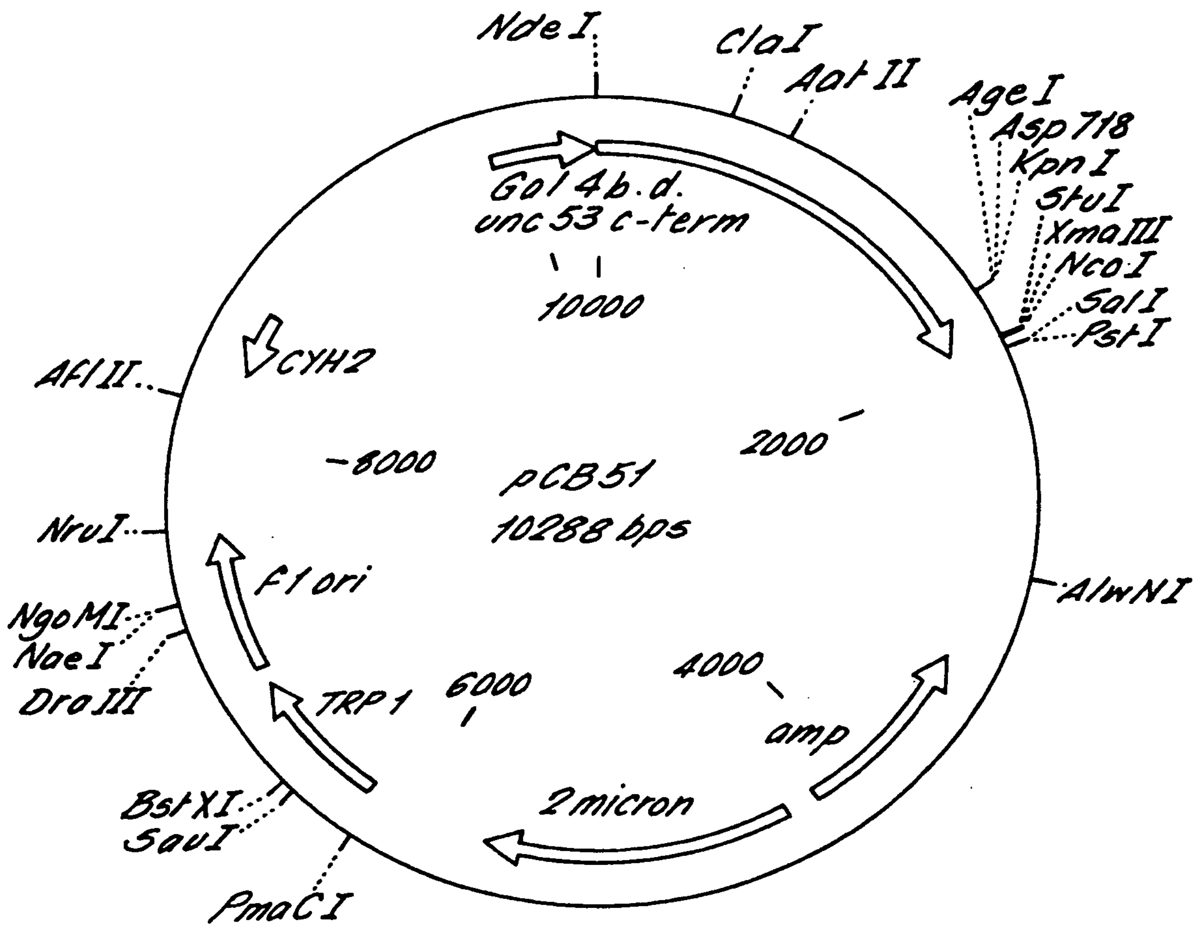
Nde I
Cla I
Aat II
Age I
Asp 718
Kpn I
Stu I
Xma III
Nco I
Sal I
Pst I
Gal 4b.d.
unc 53 c-term
10000
CYH2
Afl II
2000
-8000
pCB51
10288 bps
Nru I
AlwNI
f1 ori
Ngo MI
Nae I
Dra III
4000
6000
TRP 1
amp
2 micron
BstXI
Sau I
PmaC I

FIG. 32.

TATGCCATCA	ATTTCCGGAT	CTCAAGGAAC	TCTTGACAAC	ATTGATGTGA	TTGAGTTGAA	60
GCAAGAGCTC	AAAGAACGCG	ATAGTGCACT	TTACGAAGTC	CGCCTTGACA	ATCTGGATCG	120
TGCCCGCGAA	GTTGATGTTC	TGAGGGAGAC	AGTGAACAAG	TTGAAAACCG	AGAACAAGCA	180
ATTAAAGAAA	GAAGTGGACA	AACTCACCAA	CGGTCCAGCC	ACTCGTGCTT	CTTCCCGCGC	240
CTCAATTCCA	GTTATCTACG	ACGATGAGCA	TGTCTATGAT	GCAGCGTGTA	GCAGTACATC	300
AGCTAGTCAA	TCTTCGAAAC	GATCCTCTGG	CTGCAACTCA	ATCAAGGTTA	CTGTAAACGT	360
GGACATCGCT	GGAGAAATCA	GTTCGATCGT	TAACCCGGAC	AAAGAGATAA	TCGTAGGATA	420
TCTTGCCATG	TCAACCAGTC	AGTCATGCTG	GAAAGACATT	GATGTTTCTA	TTCTAGGACT	480
ATTTGAAGTC	TACCTATCCA	GAATTGATGT	GGAGCATCAA	CTTGGAATCG	ATGCTCGTGA	540

FIG. 32 CONTINUED. 75/99

TTCTATCCTT GGCTATCAAA TTGGTGAACT TCGACGCGTC ATTGGAGACT CCACAACCAT 600
GATAACCAGC CATCCAACTG ACATTCTTAC TTCCTCAACT ACAATCCGAA TGTTCATGCA 660
CGGTGCCGCA CAGAGTCGCG TAGACAGTCT GGTCCTTGAT ATGCTTCTTC CAAAGCAAAT 720
GATTCTCCAA CTCGTCAAGT CAATTTTGAC AGAGAGACGT CTGGTGTTAG CTGGAGCAAC 780
TGGAATTGGA AAGAGCAAAC TGGCGAAGAC CCTGGCTGCT TATGTATCTA TTCGAACAAA 840
TCAATCCGAA GATAGTATTG TTAATATCAG CATTCCTGAA AACAATAAAG AAGAATTGCT 900
TCAAGTGGAA CGACGCCTGG AAAAGATCTT GAGAAGCAAA GAATCATGCA TCGTAATTCT 960
AGATAATATC CCAAAGAATC GAATTGCATT TGTTGTATCC GTTTTTGCAA ATGTCCCACT 1020
TCAAAACAAC GAAGGTCCAT TTGTAGTATG CACAGTCAAC CGATATCAAA TCCCTGAGCT 1080
TCAAATTCAC CACAATTTCA AAATGTCAGT AATGTCGAAT CGTCTCGAAG GATTCATCCT 1140
ACGTTACCTC CGACGACGGG CGGTAGAGGA TGAGTATCGT CTAACTGTAC AGATGCCATC 1200
AGAGCTCTTC AAAATCATTG ACTTCTTCCC AATAGCTCTT CAGGCCGTCA ATAATTTTAT 1260
TGAGAAAACG AATTCTGTTG ATGTGACAGT TGGTCCAAGA GCATGCTTGA ACTGTCCTCT 1320
AACTGTCGAT GGATCCCGTG AATGGTTCAT TCGATTGTGG AATGAGAACT TCATTCCATA 1380
TTTGGAACGT GTTGCTAGAG ATGGCAAAAA AACCTTCGGT CGCTGCACTT CCTTCGAGGA 1440
TCCCACCGAC ATCGTCTCTA AAAAATGGCC GTGGTTCGAT GGTGAAAACC CGGAGAATGT 1500
GCTCAAACGT CTTCAACTCC AAGACCTCGT CCCGTCACCT GCCAACTCAT CCCGACAACA 1560
CTTCAATCCC CTCGAGTCGT TGATCCAATT GCATGCTACC AAGCATCAGA CCATCGACAA 1620
CATTTGAACA GAAGACTCTA ATCTTCTCTC GCCTCTCCCC CGCTTTCCTT ATCTTCGTAC 1680
CGGTACCTGA TGATTCCCCA TTTTCCCCCT TTTCCCCCCA ATTTCCCAGA ACCTCCTGTT 1740
CCCTTTGTTC CTAGTCCTCC CGGGTGCCGA CGCCGAAGCG ATTTAAAAAC CTTTTTCTTT 1800
CCGAAACATT TCCCATTGCT CATTAATAGT CAAATTGAAT AAACAGTGTA TGTACTTAAA 1860
AAAAAAAAAA AAAAAAAAAA AAAAGGCCTA TGCGGCCGGG CCATGGAGGC CGAATTCCCG 1920
GGGATCCGTC GACCTGCAGC CAAGCTAATT CCGGGCGAAT TTCTTATGAT TTATGATTTT 1980
TATTATTAAA TAAGTTATAA AAAAAATAAG TGTATACAAA TTTTAAAGTG ACTCTTAGGT 2040
TTTAAAACGA AAATTCTTGT TCTTGAGTAA CTCTTTCCTG TAGGTCAGGT TGCTTTCTCA 2100
GGTATAGCAT GAGGTCGCTC TTATTGACCA CACCTCTACC GGCATGCAAG CTTGGCGTAA 2160
TCATGGTCAT AGCTGTTTCC TGTGTGAAAT TGTTATCCGC TCACAATTCC ACACAACATA 2220
CGAGCCGGAA GCATAAAGTG TAAAGCCTGG GGTGCCTAAT GAGTGAGGTA ACTCACATTA 2280
ATTGCGTTGC GCTCACTGCC CGCTTTCCAG TCGGGAAACC TGTCGTGCCA GCTGGATTAA 2340
TGAATCGGCC AACGCGCGGG GAGAGGCGGT TTGCGTATTG GGCGCTCTTC CGCTTCCTCG 2400
CTCACTGACT CGCTGCGCTC GGTCGTTCGG CTGCGGCGAG CGGTATCAGC TCACTCAAAG 2460

FIG. 32 CONTINUED 76/99

GCGGTAATAC GGTTATCCAC AGAATCAGGG GATAACGCAG GAAAGAACAT GTGAGCAAAA 2520
GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT CCATAGGCTC 2580
CGCCCCCCTG ACGAGCATCA CAAAAATCGA CGCTCAAGTC AGAGGTGGCG AAACCCGACA 2640
GGACTATAAA GATACCAGGC GTTTCCCCCT GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG 2700
ACCCTGCCGC TTACCGGATA CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT 2760
CATAGCTCAC GCTGTAGGTA TCTCAGTTCG GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT 2820
GTGCACGAAC CCCCCGTTCA GCCCGACCGC TGCGCCTTAT CCGGTAACTA TCGTCTTGAG 2880
TCCAACCCGG TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC 2940
AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA CTACGGCTAC 3000
ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC CAGTTACCTT CGGAAAAAGA 3060
GTTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC 3120
AAGCAGCAGA TTACGCGCAG AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG 3180
GGGTCTGACG CTCAGTGGAA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT GAGATTATCA 3240
AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC AATCTAAAGT 3300
ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA TCAGTGAGGC ACCTATCTCA 3360
GCGATCTGTC TATTTCGTTC ATCCATAGTT GCCTGACTCC CCGTCGTGTA GATAACTACG 3420
ATACGGGAGG GCTTACCATC TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA 3480
CCGGCTCCAG ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT 3540
CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT 3600
AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGCCATTG CTACAGGCAT CGTGGTGTCA 3660
CGCTCGTCGT TTGGTATGGC TTCATTCAGC TCCGGTTCCC AACGATCAAG GCGAGTTACA 3720
TGATCCCCCA TGTTGTGCAA AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA 3780
AGTAAGTTGG CCGCAGTGTT ATCACTCATG GTTATGGCAG CACTGCATAA TTCTCTTACT 3840
GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA GTCATTCTGA 3900
GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT CAATACGGGA TAATACCGCG 3960
CCACATAGCA GAACTTTAAA AGTGCTCATC ATTGGAAAAC GTTCTTCGGG GCGAAAACTC 4020
TCAAGGATCT TACCGCTGTT GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA 4080
TCTTCAGCAT CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT 4140
GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT CTTCCTTTTT 4200
CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT ATTTGAATGT 4260
ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTC CCCGAAAAGT GCCACCTGAA 4320
CGAAGCATCT GTGCTTCATT TTGTAGAACA AAAATGCAAC GCGAGAGCGC TAATTTTTCA 4380

FIG. 32 CONTINUED. 77/99

AACAAAGAAT CTGAGCTGCA TTTTTACAGA ACAGAAATGC AACGCGAAAG CGCTATTTTA 4440
CCAACGAAGA ATCTGTGCTT CATTTTTGTA AAACAAAAAT GCAACGCGAG AGCGCTAATT 4500
TTTCAAACAA AGAATCTGAG CTGCATTTTT ACAGAACAGA AATGCAACGC GAGAGCGCTA 4560
TTTTACCAAC AAAGAATCTA TACTTCTTTT TTGTTCTACA AAAATGCATC CCGAGAGCGC 4620
TATTTTTCTA ACAAAGCATC TTAGATTACT TTTTTTCTCC TTTGTGCGCT CTATAATGCA 4680
GTCTCTTGAT AACTTTTTGC ACTGTAGGTC CGTTAAGGTT AGAAGAAGGC TACTTTGGTG 4740
TCTATTTTCT CTTCCATAAA AAAAGCCTGA CTCCACTTCC CGCGTTTACT GATTACTAGC 4800
GAAGCTGCGG GTGCATTTTT TCAAGATAAA GGCATCCCCG ATTATATTCT ATACCGATGT 4860
GGATTGCGCA TACTTTGTGA ACAGAAAGTG ATAGCGTTGA TGATTCTTCA TTGGTCAGAA 4920
AATTATGAAC GGTTTCTTCT ATTTTGTCTC TATATACTAC GTATAGGAAA TGTTTACATT 4980
TTCGTATTGT TTTCGATTCA CTCTATGAAT AGTTCTTACT ACAATTTTTT TGTCTAAAGA 5040
GTAATACTAG AGATAAACAT AAAAAATGTA GAGGTCGAGT TTAGATGCAA GTTCAAGGAG 5100
CGAAAGGTGG ATGGGTAGGT TATATAGGGA TATAGCACAG AGATATATAG CAAAGAGATA 5160
CTTTTGAGCA ATGTTTGTGG AAGCGGTATT CGCAATATTT TAGTAGCTCG TTACAGTCCG 5220
GTGCGTTTTT GGTTTTTTGA AAGTGCGTCT TCAGAGCGCT TTTGGTTTTC AAAAGCGCTC 5280
TGAAGTTCCT ATACTTTCTA GAGAATAGGA ACTTCGGAAT AGGAACTTCA AAGCGTTTCC 5340
GAAAACGAGC GCTTCCGAAA ATGCAACGCG AGCTGCGCAC ATACAGCTCA CTGTTCACGT 5400
CGCACCTATA TCTGCGTGTT GCCTGTATAT ATATATACAT GAGAAGAACG GCATAGTGCG 5460
TGTTTATGCT TAAATGCGTA CTTATATGCG TCTATTTATG TAGGATGAAA GGTAGTCTAG 5520
TACCTCCTGT GATATTATCC CATTCCATGC GGGGTATCGT ATGCTTCCTT CAGCACTACC 5580
CTTTAGCTGT TCTATATGCT GCCACTCCTC AATTGGATTA GTCTCATCCT TCAATGCTAT 5640
CATTTCCTTT GATATTGGAT CATATTAAGA AACCATTATT ATCATGACAT TAACCTATAA 5700
AAATAGGCGT ATCACGAGGC CCTTTCGTCT CGCGCGTTTC GGTGATGACG GTGAAAACCT 5760
CTGACACATG CAGCTCCCGG AGACGGTCAC AGCTTGTCTG TAAGCGGATG CCGGGAGCAG 5820
ACAAGCCCGT CAGGGCGCGT CAGCGGGTGT TGGCGGGTGT CGGGGCTGGC TTAACTATGC 5880
GGCATCAGAG CAGATTGTAC TGAGAGTGCA CCATAGATCA ACGACATTAC TATATATATA 5940
ATATAGGAAG CATTTAATAG ACAGCATCGT AATATATGTG TACTTTGCAG TTATGACGCC 6000
AGATGGCAGT AGTGGAAGAT ATTCTTTATT GAAAAATAGC TTGTCACCTT ACGTACAATC 6060
TTGATCCGGA GCTTTTCTTT TTTTGCCGAT TAAGAATTAA TTCGGTCGAA AAAAGAAAAG 6120
GAGAGGGCCA AGAGGGAGGG CATTGGTGAC TATTGAGCAC GTGAGTATAC GTGATTAAGC 6180
ACACAAAGGC AGCTTGGAGT ATGTCTGTTA TTAATTTCAC AGGTAGTTCT GGTCCATTGG 6240
TGAAAGTTTG CGGCTTGCAG AGCACAGAGG CCGCAGAATG TGCTCTAGAT TCCGATGCTG 6300

FIG. 32 CONTINUED. 78/99

ACTTGCTGGG TATTATATGT GTGCCCAATA GAAAGAGAAC AATTGACCCG GTTATTGCAA 6360
GGAAAATTTC AAGTCTTGTA AAAGCATATA AAAATAGTTC AGGCACTCCG AAATACTTGG 6420
TTGGCGTGTT TCGTAATCAA CCTAAGGAGG ATGTTTTGGC TCTGGTCAAT GATTACGGCA 6480
TTGATATCGT CCAACTGCAT GGAGATGAGT CGTGGCAAGA ATACCAAGAG TTCCTCGGTT 6540
TGCCAGTTAT TAAAAGACTC GTATTTCCAA AAGACTGCAA CATACTACTC AGTGCAGCTT 6600
CACAGAAACC TCATTCGTTT ATTCCCTTGT TTGATTCAGA AGCAGGTGGG ACAGGTGAAC 6660
TTTTGGATTG GAACTCGATT TCTGACTGGG TTGGAAGGCA AGAGAGCCCC GAAAGCTTAC 6720
ATTTTATGTT AGCTGGTGGA CTGACGCCAG AAAATGTTGG TGATGCGCTT AGATTAAATG 6780
GCGTTATTGG TGTTGATGTA AGCGGAGGTG TGGAGACAAA TGGTGTAAAA GACTCTAACA 6840
AAATAGCAAA TTTCGTCAAA AATGCTAAGA AATAGGTTAT TACTGAGTAG TATTTATTTA 6900
AGTATTGTTT GTGCACTTGC CGATCTATGC GGTGTGAAAT ACCGCACAGA TGCGTAAGGA 6960
GAAAATACCG CATCAGGAAA TTGTAAACGT TAATATTTTG TTAAAATTCG CGTTAAATTT 7020
TTGTTAAATC AGCTCATTTT TTAACCAATA GGCCGAAATC GGCAAAATCC CTTATAAATC 7080
AAAAGAATAG ACCGAGATAG GGTTGAGTGT TGTTCCAGTT TGGAACAAGA GTCCACTATT 7140
AAAGAACGTG GACTCCAACG TCAAAGGGCG AAAAACCGTC TATCAGGGCG ATGGCCCACT 7200
ACGTGAACCA TCACCCTAAT CAAGTTTTTT GGGGTCGAGG TGCCGTAAAG CACTAAATCG 7260
GAACCCTAAA GGGAGCCCCC GATTTAGAGC TTGACGGGGA AAGCCGGCGA ACGTGGCGAG 7320
AAAGGAAGGG AAGAAAGCGA AAGGAGCGGG CGCTAGGGCG CTGGCAAGTG TAGCGGTCAC 7380
GCTGCGCGTA ACCACCACAC CCGCCGCGCT TAATGCGCCG CTACAGGGCG CGTCGCGCCA 7440
TTCGCCATTC AGGCTGCGCA ACTGTTGGGA AGGGCGATCG GTGCGGGCCT CTTCGCTATT 7500
ACGCCAGCTG GCGAAAGGGG GATGTGCTGC AAGGCGATTA AGTTGGGTAA CGCCAGGGTT 7560
TTCCCAGTCA CGACGTTGTA AAACGACGGC CAGTCGTCCA AGCTTTCGCG AGCTCGAGAT 7620
CCCGAGCTTT GCAAATTAAA GCCTTCGAGC GTCCCAAAAC CTTCTCAAGC AAGGTTTTCA 7680
GTATAATGTT ACATGCGTAC ACGCGTCTGT ACAGAAAAAA AAGAAAAATT TGAAATATAA 7740
ATAACGTTCT TAATACTAAC ATAACTATAA AAAAATAAAT AGGGACCTAG ACTTCAGGTT 7800
GTCTAACTCC TTCCTTTTCG GTTAGAGCGG ATGTGGGGGG AGGGCGTGAA TGTAAGCGTG 7860
ACATAACTAA TTACATGATA TCGACAAAGG AAAAGGGGCC TGTTTACTCA CAGGCTTTTT 7920
TCAAGTAGGT AATTAAGTCG TTTCTGTCTT TTTCCTTCTT CAACCCACCA AAGGCCATCT 7980
TGGTACTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT 8040
TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT CATAGAAATA ATACAGAAGT 8100
AGATGTTGAA TTAGATTAAA CTGAAGATAT ATAATTTATT GGAAAATACA TAGAGCTTTT 8160
TGTTGATGCG CTTAAGCGAT CAATTCAACA ACACCACCAG CAGCTCTGAT TTTTTCTTCA 8220

FIG. 32 CONTINUED. 79/99

GCCAACTTGG AGACGAATCT AGCTTTGACG ATAACTGGAA CATTTGGGAT TCTACCCTTA 8280
CCCAAGATCT TACCGTAACC GGCTGCCAAA GTGTCAATAA CTGGAGCAGT TTCCTTAGAA 8340
GCAGATTTCA AGTATTGGTC TCTCTTGTCT TCTGGGATCA ATGTCCACAA TTTGTCCAAG 8400
TTCAAGACTG GCTTCCAGAA ATGAGCTTGT TGCTTGTGGA AGTATCTCAT ACCAANCCTT 8460
ACCGAAATAA CCTGGATGGT ATTTATCCAT GTTAATTCTG TGGTGATGTT GACCACCGGC 8520
CATACCTCTA CCACCGGGGT GCTTTCTGTG CTTACCGATA CGACCTTTAC CGGCTGAGAC 8580
GTGACCTCTG TGCTTTCTAG TCTTAGTGAA TCTGGAAGGC ATTCTTGATT AGTTGGATGA 8640
TTGTTCTGGG ATTTAATGCA AAAAAATCAC TAAGAAGGAA AAAAATCAAC GGAGAAAGCA 8700
AACGCCATCT TAAATATACG GGATACAGAT GAAAGGTTTG AACCTATCTG GGAAAATACG 8760
CATTAAACAA GCGAAAAACT GCGAGGAAAA TTGTTTGCGT CTCTGCGGGC TATTCACGCG 8820
CCAGAGGAAA ATAGGAAAAA TAACAGGGCA TTAGAAAAAT AATTTTGATT TTGGTAATGT 8880
GTGGGTCCCT GGTGTACAGA TGTTACATTG GTTACAGTAC TCTTGTTTTT GCTGTGTTTT 8940
TCGATGAATC TCCAAAATGG TTGTTAGCAC ATGGAAGAGT CACCGATGCT AAGTTATCTC 9000
TATGTAAGCT ACGTGGCGTG ACTTTTGATG AAGCCGCACA AGAGATACAG GATTGGCAAC 9060
TGCAAATAGA ATCTGGGGAT CTAGATATCC TTTTGTTGTT TCCGGGTGTA CAATATGGAC 9120
TTCCTCTTTT CTGGCAACCA AACCCATACA TCGGGATTCC TATAATACCT TCGTTGGTCT 9180
CCCTAACATG TAGGTGGCGG AGGGGAGATA TACAATAGAA CAGATACCAG ACAAGACATA 9240
ATGGGCTAAA CAAGACTACA CCAATTACAC TGCCTCATTG ATGGTGGTAC ATAACGAACT 9300
AATACTGTAG CCCTAGACTT GATAGCCATC ATCATATCGA AGTTTCACTA CCCTTTTTCC 9360
ATTTGCCATC TATTGAAGTA ATAATAGGCG CATGCAACTT CTTTTCTTTT TTTTTCTTTT 9420
CTCTCTCCCC CGTTGTTGTC TCACCATATC CGCAATGACA AAAAAAATGA TGGAAGACAC 9480
TAAAGGAAAA AATTAACGAC AAAGACAGCA CCAACAGATG TCGTTGTTCC AGAGCTGATG 9540
AGGGGTATCT TCGAACACAC GAAACTTTTT CCTTCCTTCA TTCACGCACA CTACTCTCTA 9600
ATGAGCAACG GTATACGGCC TTCCTTCCAG TTACTTGAAT TTGAAATAAA AAAAGTTTGC 9660
CGCTTTGCTA TCAAGTATAA ATAGACCTGC AATTATTAAT CTTTTGTTTC CTCGTCATTG 9720
TTCTCGTTCC CTTTCTTCCT TGTTTCTTTT TCTGCACAAT ATTTCAAGCT ATACCAAGCA 9780
TACAATCAAC TCCAAGCTTG AAGCAAGCCT CCTGAAAGAT GAAGCTACTG TCTTCTATCG 9840
AACAAGCATG CGATATTTGC CGACTTAAAA AGCTCAAGTG CTCCAAAGAA AAACCGAAGT 9900
GCGCCAAGTG TCTGAAGAAC AACTGGGAGT GTCGCTACTC TCCCAAAACC AAAAGGTCTC 9960
CGCTGACTAG GGCACATCTG ACAGAAGTGG AATCAAGGCT AGAAAGACTG GAACAGCTAT 10020
TTCTACTGAT TTTTCCTCGA GAAGACCTTG ACATGATTTT GAAAATGGAT TCTTTACAGG 10080
ATATAAAAGC ATTGTTAACA GGATTATTTG TACAAGATAA TGTGAATAAA GATGCCGTCA 10140

FIG. 32 CONTINUED.

CAGATAGATT GGCTTCAGTG GAGACTGATA TGCCTCTAAC ATTGAGACAG CATAGAATAA 10200
GTGCGACATC ATCATCGGAA GAGAGTAGTA ACAAAGGTCA AAGACAGTTG ACTGTATCGC 10260
CGGAATTGCA ATACCCAGCT TTGACTCA 10288

FIG. 33

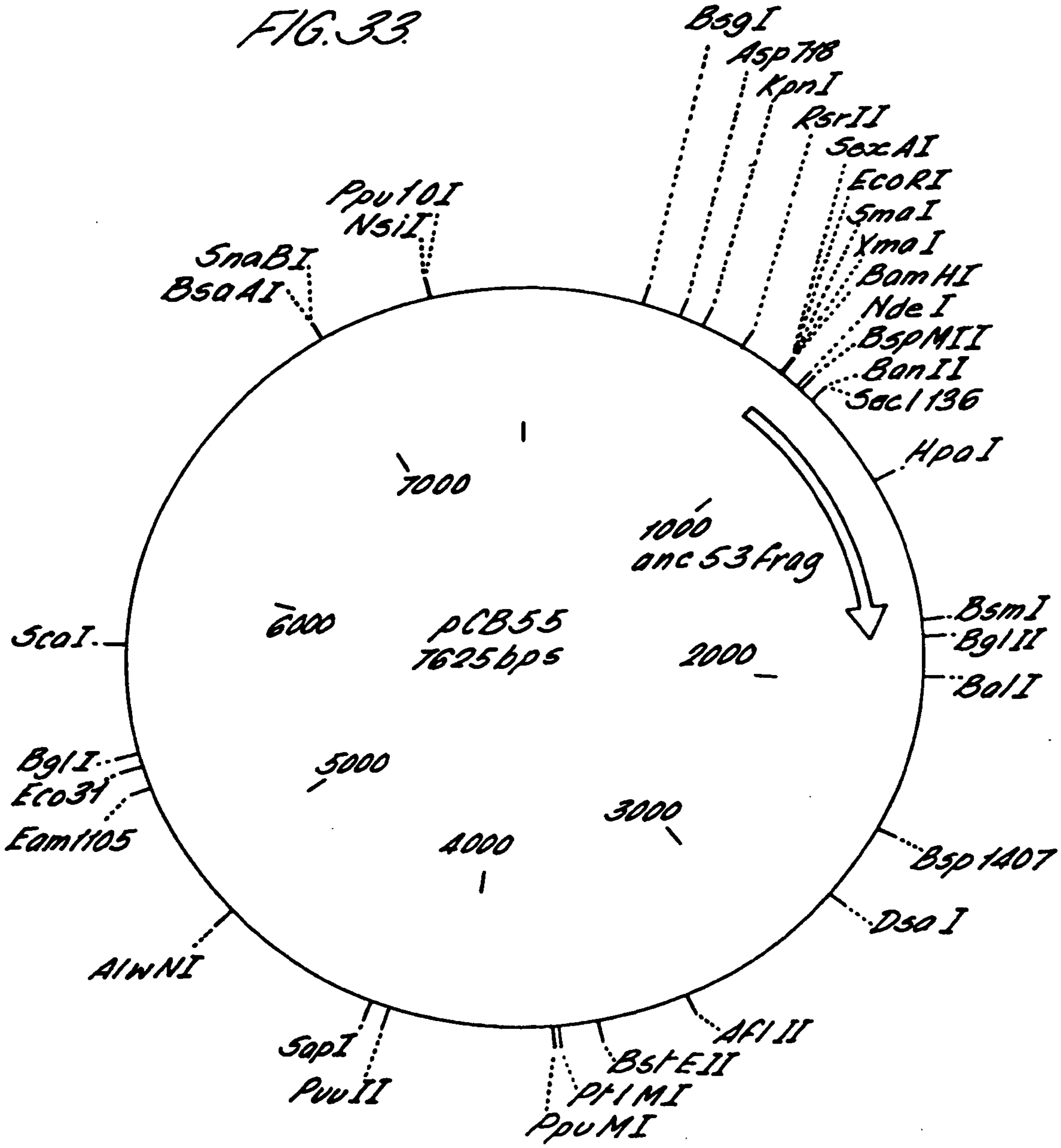
BsgI
Asp718
KpnI
RsrII
SexAI
EcoRI
SmaI
XmaI
BamHI
NdeI
BspMII
BanII
Sac I 136
HpaI
BsmI
BglII
BalI
Bsp1407
DsaI
AflII
BstEII
PflMI
PpuMI
PvuII
SapI
AlwNI
Eam1105
Eco31
BglI
ScaI
BsaAI
SnaBI
Ppu10I
NsiI
7000
1000
anc53 frag
6000
pCB55
7625 bps
2000
5000
3000
4000

FIG. 34.

GCTTGCATGC AACTTCTTTT CTTTTTTTTT CTTTTCTCTC TCCCCCGTTG TTGTCTCACC 60
ATATCCGCAA TGACAAAAAA AATGATGGAA GACACTAAAG GAAAAAATTA ACGACAAAGA 120
CAGCACCAAC AGATGTCGTT GTTCCAGAGC TGATGAGGGG TATCTTCGAA CACACGAAAC 180
TTTTTCCTTC CTTCATTCAC GCACACTACT CTCTAATGAG CAACGGTATA CGGCCTTCCT 240
TCCAGTTACT TGAATTTGAA ATAAAAAAAG TTTGCCGCTT TGCTATCAAG TATAAATAGA 300
CCTGCAATTA TTAATCTTTT GTTTCCTCGT CATTGTTCTC GTTCCCTTTC TTCCTTGTTT 360
CTTTTTCTGC ACAATATTTC AAGCTATACC AAGCATACAA TCAACTCCAA GCTTTGCAAA 420
GATGGATAAA GCGGAATTAA TTCCCGAGCC TCCAAAAAAG AAGAGAAAGG TCGAATTGGG 480
TACCGCCGCC AATTTTAATC AAAGTGGGAA TATTGCTGAT AGCTCATTGT CCTTCACTTT 540
CACTAACAGT AGCAACGGTC CGAACCTCAT AACAACTCAA ACAAATTCTC AAGCGCTTTC 600
ACAACCAATT GCCTCCTCTA ACGTTCATGA TAACTTCATG AATAATGAAA TCACGGCTAG 660
TAAAATTGAT GATGGTAATA ATTCAAAACC ACTGTCACCT GGTTGGACGG ACCAAACTGC 720
GTATAACGCG TTTGGAATCA CTACAGGGAT GTTTAATACC ACTACAATGG ATGATGTATA 780
TAACTATCTA TTCGATGATG AAGATACCCC ACCAAACCCA AAAAAAGAGA TCGAATTCCC 840
GGGGATCCGC TCCTCACTCT CCAAGTTCAC CAAGAAGAAG AACAAGAACT ACGACGAAGC 900
ACATATGCCA TCAATTTCCG GATCTCAAGG AACTCTTGAC AACATTGATG TGATTGAGTT 960
GAAGCAAGAG CTCAAAGAAC GCGATAGTGC ACTTTACGAA GTCCGCCTTG ACAATCTGGA 1020
TCGTGCCCGC GAAGTTGATG TTCTGAGGGA GACAGTGAAC AAGTTGAAAA CCGAGAACAA 1080
GCAATTAAAG AAAGAAGTGG ACAAACTCAC CAACGGTCCA GCCACTCGTG CTTCTTCCCG 1140
CGCCTCAATT CCAGTTATCT ACGACGATGA GCATGTCTAT GATGCAGCGT GTAGCAGTAC 1200

FIG. 34 CONTINUED. 83/99

ATCAGCTAGT CAATCTTCGA AACGATCCTC TGGCTGCAAC TCAATCAAGG TTACTGTAAA 1260

CGTGGACATC GCTGGAGAAA TCAGTTCGAT CGTTAACCCG GACAAAGAGA TAATCGTAGG 1320

ATATCTTGCC ATGTCAACCA GTCAGTCATG CTGGAAAGAC ATTGATGTTT CTATTCTAGG 1380

ACTATTTGAA GTCTACCTAT CCAGAATTGA TGTGGAGCAT CAACTTGGAA TCGATGCTCG 1440

TGATTCTATC CTTGGCTATC AAATTGGTGA ACTTCGACGC GTCATTGGAG ACTCCACAAC 1500

CATGATAACC AGCCATCCAA CTGACATTCT TACTTCCTCA ACTACAATCC GAATGTTCAT 1560

GCACGGTGCC GCACAGAGTC GCGTAGACAG TCTGGTCCTT GATATGCTTC TTCCAAAGCA 1620

AATGATTCTC CAACTCGTCA AGTCAATTTT GACAGAGAGA CGTCTGGTGT TAGCTGGAGC 1680

AACTGGAATT GGAAAGAGCA AACTGGCGAA GACCCTGGCT GCTTATGTAT CTATTCGAAC 1740

AAATCAATCC GAAGATAGTA TTGTTAATAT CAGCATTCCT GAAAACAATA AAGAAGAATT 1800

GCTTCAAGTG GAACGACGCC TGGAAAAGAT CTATGAATCG TAGATACTGA AAAACCCCGC 1860

AAGTTCACTT CAACTGTGCA TCGTGCACCA TCTCAATTTC TTTCATTTAT ACATCGTTTT 1920

GCCTTCTTTT ATGTAACTAT ACTCCTCTAA GTTTCAATCT TGGCCATGTA ACCTCTGATC 1980

TATAGAATTT TTTAAATGAC TAGAATTAAT GCCCATCTTT TTTTTGGACC TAAATTCTTC 2040

ATGAAAATAT ATTACGAGGG CTTATTCAGA AGCTTTGGAC TTCTTCGCCA GAGGTTTGGT 2100

CAAGTCTCCA ATCAAGGTTG TCGGCTTGTC TACCTTGCCA GAAATTTACG AAAAGATGGA 2160

AAAGGGTCAA ATCGTTGGTA GATACGTTGT TGACACTTCT AAATAAGCGA ATTTCTTATG 2220

ATTTATGATT TTTATTATTA AATAAGTTAT AAAAAAAATA AGTGTATACA AATTTTAAAG 2280

TGACTCTTAG GTTTTAAAAC GAAAATTCTT GTTCTTGAGT AACTCTTTCC TGTAGGTCAG 2340

GTTGCTTTCT CAGGTATAGC ATGAGGTCGC TCTTATTGAC CACACCTCTA CCGGCATGCC 2400

CGAAATTCCC CTACCCTATG AACATATTCC ATTTTGTAAT TTCGTGTCGT TTCTATTATG 2460

AATTTCATTT ATAAAGTTTA TGTACAAATA TCATAAAAAA AGAGAATCTT TTTAAGCAAG 2520

GATTTTCTTA ACTTCTTCGG CGACAGCATC ACCGACTTCG GTGGTACTGT TGGAACCACC 2580

TAAATCACCA GTTCTGATAC CTGCATCCAA AACCTTTTTA ACTGCATCTT CAATGGCCTT 2640

ACCTTCTTCA GGCAAGTTCA ATGACAATTT CAACATCATT GCAGCAGACA AGATAGTGGC 2700

GATAGGGTCA ACCTTATTCT TTGGCAAATC TGGAGCAGAA CCGTGGCATG GTTCGTACAA 2760

ACCAAATGCG GTGTTCTTGT CTGGCAAAGA GGCCAAGGAC GCAGATGGCA ACAAACCCAA 2820

GGAACCTGGG ATAACGGAGG CTTCATCGGA GATGATATCA CCAAACATGT TGCTGGTGAT 2880

TATAATACCA TTTAGGTGGG TTGGGTTCTT AACTAGGATC ATGGCGGCAG AATCAATCAA 2940

TTGATGTTGA ACCTTCAATG TAGGAAATTC GTTCTTGATG GTTTCCTCCA CAGTTTTTCT 3000

CCATAATCTT GAAGAGGCCA AAACATTAGC TTTATCCAAG GACCAAATAG GCAATGGTGG 3060

CTCATGTTGT AGGGCCATGA AAGCGGCCAT TCTTGTGATT CTTTGCACTT CTGGAACGGT 3120

FIG. 34 CONTINUED. 84/99

GTATTGTTCA CTATCCCAAG CGACACCATC ACCATCGTCT TCCTTTCTCT TACCAAAGTA 3180
AATACCTCCC ACTAATTCTC TGACAACAAC GAAGTCAGTA CCTTTAGCAA ATTGTGGCTT 3240
GATTGGAGAT AAGTCTAAAA GAGAGTCGGA TGCAAAGTTA CATGGTCTTA AGTTGGCGTA 3300
CAATTGAAGT TCTTTACGGA TTTTTAGTAA ACCTTGTTCA GGTCTAACAC TACCTGTACC 3360
CCATTTAGGA CCACCCACAG CACCTAACAA AACGGCATCA ACCTTCTTGG AGGCTTCCAG 3420
CGCCTCATCT GGAAGTGGGA CACCTGTAGC ATCGATAGCA GCACCACCAA TTAAATGATT 3480
TTCGAAATCG AACTTGACAT TGGAACGAAC ATCAGAAATA GCTTTAAGAA CCTTAATGGC 3540
TTCGGCTGTG ATTTCTTGAC CAACGTGGTC ACCTGGCAAA ACGACGATCT TCTTAGGGGC 3600
AGACATTAGA ATGGTATATC CTTGAAATAT ATATATATAT TGCTGAAATG TAAAAGGTAA 3660
GAAAAGTTAG AAAGTAAGAC GATTGCTAAC CACCTATTGG AAAAAACAAT AGGTCCTTAA 3720
ATAATATTGT CAACTTCAAG TATTGTGATG CAAGCATTTA GTCATGAACG CTTCTCTATT 3780
CTATATGAAA AGCCGGTTCC GGCCTCTCAC CTTTCCTTTT TCTCCCAATT TTTCAGTTGA 3840
AAAAGGTATA TGCGTCAGGC GACCTCTGAA ATTAACAAAA AATTTCCAGT CATCGAATTT 3900
GATTCTGTGC GATAGCGCCC CTGTGTGTTC TCGTTATGTT GAGGAAAAAA ATAATGGTTG 3960
CTAAGAGATT CGAACTCTTG CATCTTACGA TACCTGAGTA TTCCCACAGT TGGGGATCTC 4020
GACTCTAGCT AGAGGATCAA TTCGTAATCA TGGTCATAGC TGTTTCCTGT GTGAAATTGT 4080
TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT 4140
GCCTAATGAG TGAGGTAACT CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG 4200
GGAAACCTGT CGTGCCAGCT GGATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG 4260
CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT CGTTCGGCTG 4320
CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT 4380
AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC 4440
GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC 4500
TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT TCCCCCTGGA 4560
AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT 4620
CTCCCTTCGG GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT CAGTTCGGTG 4680
TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG CACGAACCCC CCGTTCAGCC CGACCGCTGC 4740
GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG 4800
GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC 4860
TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG TATTTGGTAT CTGCGCTCTG 4920
CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT GATCCGGCAA ACAAACCACC 4980
GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA CGCGCAGAAA AAAAGGATCT 5040

FIG. 34 CONTINUED. 85/99

CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT 5100
TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA 5160
AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA CTTGGTCTGA CAGTTACCAA 5220
TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC CATAGTTGCC 5280
TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT 5340
GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA 5400
GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT CCGCCTCCAT CCAGTCTATT 5460
AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG CAACGTTGTT 5520
GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC 5580
GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA AGCGGTTAGC 5640
TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG CAGTGTTATC ACTCATGGTT 5700
ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT TTCTGTGACT 5760
GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GGCGACCGAG TTGCTCTTGC 5820
CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT 5880
GGAAAACGTT CTTCGGGGCG AAAACTCTCA AGGATCTTAC CGCTGTTGAG ATCCAGTTCG 5940
ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTCAC CAGCGTTTCT 6000
GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA 6060
TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA GCATTTATCA GGGTTATTGT 6120
CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCCGCGC 6180
ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA TTATTATCAT GACATTAACC 6240
TATAAAAATA GGCGTATCAC GAGGCCCTTT CGTCTCGCGC GTTTCGGTGA TGACGGTGAA 6300
AACCTCTGAC ACATGCAGCT CCCGGAGACG GTCACAGCTT GTCTGTAAGC GGATGCCGGG 6360
AGCAGACAAG CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG GGTGTCGGGG CTGGCTTAAC 6420
TATGCGGCAT CAGAGCAGAT TGTACTGAGA GTGCACCATA ACGCATTTAA GCATAAACAC 6480
GCACTATGCC GTTCTTCTCA TGTATATATA TATACAGGCA ACACGCAGAT ATAGGTGCGA 6540
CGTGAACAGT GAGCTGTATG TGCGCAGCTC GCGTTGCATT TTCGGAAGCG CTCGTTTTCG 6600
GAAACGCTTT GAAGTTCCTA TTCCGAAGTT CCTATTCTCT AGCTAGAAAG TATAGGAACT 6660
TCAGAGCGCT TTTGAAAACC AAAAGCGCTC TGAAGACGCA CTTTCAAAAA ACCAAAAACG 6720
CACCGGACTG TAACGAGCTA CTAAAATATT GCGAATACCG CTTCCACAAA CATTGCTCAA 6780
AAGTATCTCT TTGCTATATA TCTCTGTGCT ATATCCCTAT ATAACCTACC CATCCACCTT 6840
TCGCTCCTTG AACTTGCATC TAAACTCGAC CTCTACATTT TTTATGTTTA TCTCTAGTAT 6900
TACTCTTTAG ACAAAAAAAT TGTAGTAAGA ACTATTCATA GAGTGAATCG AAAACAATAC 6960

FIG. 34 CONTINUED.

GAAAATGTAA	ACATTTCCTA	TACGTAGTAT	ATAGAGACAA	AATAGAAGAA	ACCGTTCATA	7020
ATTTTCTGAC	CAATGAAGAA	TCATCAACGC	TATCACTTTC	TGTTCACAAA	GTATGCGCAA	7080
TCCACATCGG	TATAGAATAT	AATCGGGGAT	GCCTTTATCT	TGAAAAAATG	CACCCGCAGC	7140
TTCGCTAGTA	ATCAGTAAAC	GCGGGAAGTG	GAGTCAGGCT	TTTTTTATGG	AAGAGAAAAT	7200
AGACACCAAA	GTAGCCTTCT	TCTAACCTTA	ACGGACCTAC	AGTGCAAAAA	GTTATCAAGA	7260
GACTGCATTA	TAGAGCGCAC	AAAGGAGAAA	AAAAGTAATC	TAAGATGCTT	TGTTAGAAAA	7320
ATAGCGCTCT	CGGGATGCAT	TTTTGTAGAA	CAAAAAAGAA	GTATAGATTC	TTTGTTGGTA	7380
AAATAGCGCT	CTCGCGTTGC	ATTTCTGTTC	TGTAAAAATG	CAGCTCAGAT	TCTTTGTTTG	7440
AAAAATTAGC	GCTCTCGCGT	TGCATTTTTG	TTTTACAAAA	ATGAAGCACA	GATTCTTCGT	7500
TGGTAAAATA	GCGCTTTCGC	GTTGCATTTC	TGTTCTGTAA	AAATGCAGCT	CAGATTCTTT	7560
GTTTGAAAAA	TTAGCGCTCT	CGCGTTGCAT	TTTTGTTCTA	CAAAATGAAG	CACAGATGCT	7620
TCGTT						7625

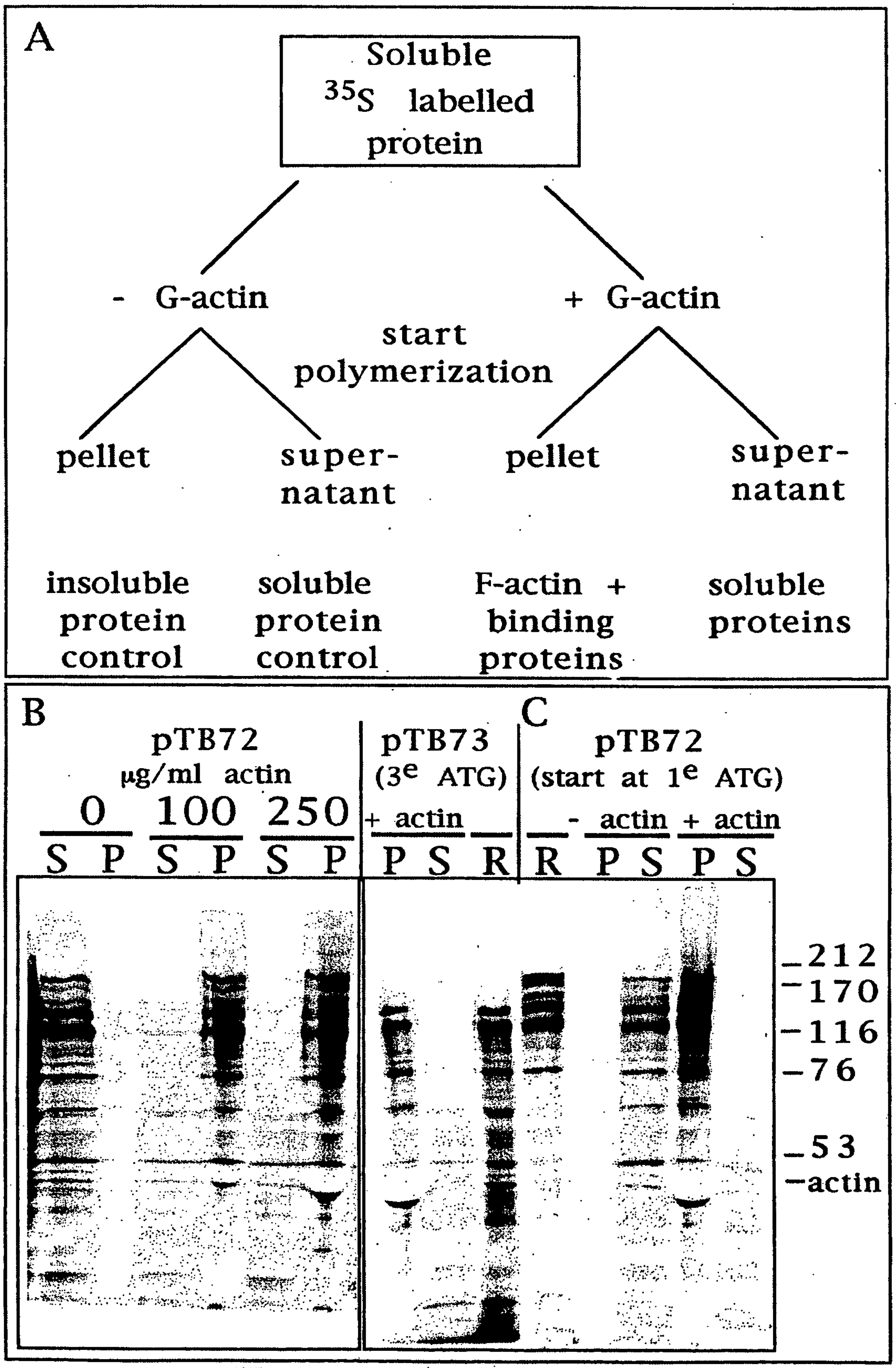
A
Soluble
35S labelled
protein
- G-actin
+ G-actin
start
polymerization
pellet
super-
natant
pellet
super-
natant
insoluble
protein
control
soluble
protein
control
F-actin +
binding
proteins
soluble
proteins
B
pTB72
µg/ml actin
0
100
250
S P S P S P
pTB73
(3e ATG)
+ actin
P S R
C
pTB72
(start at 1e ATG)
- actin
+ actin
R P S P S
212
170
116
76
53
actin

FIG. 35.

FIG. 36.

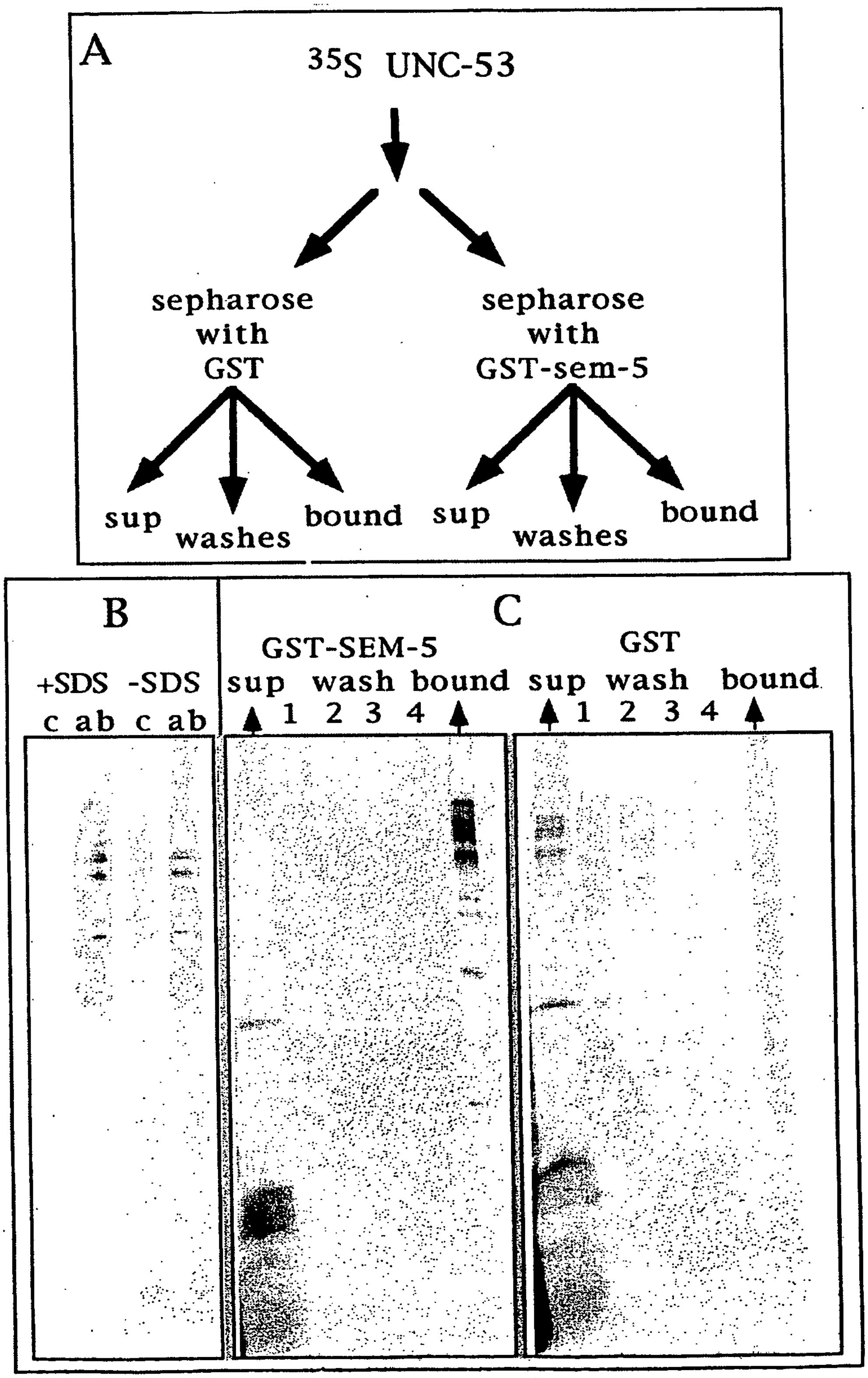
A
^{35}S UNC-53
sepharose with GST
sepharose with GST-sem-5
sup
washes
bound
sup
washes
bound
B
+SDS -SDS
c ab c ab
C
GST-SEM-5
sup wash bound
1 2 3 4
GST
sup wash bound
1 2 3 4

FIG. 36 (CONTD.)

D

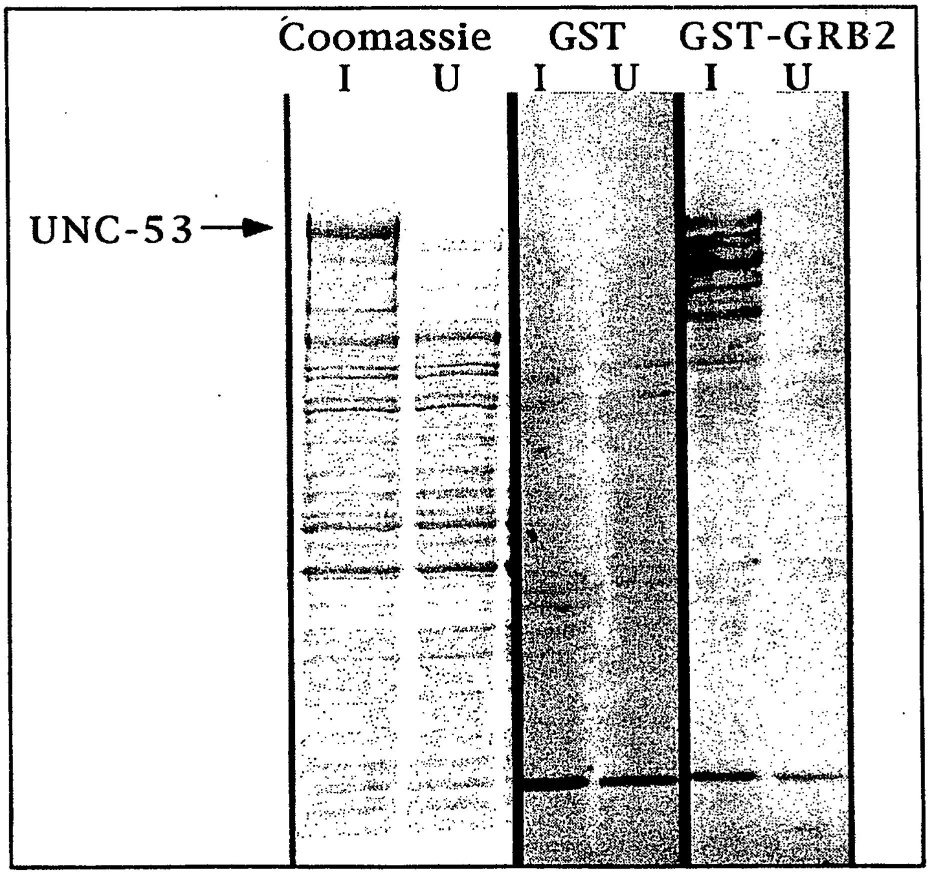

FIG. 37.

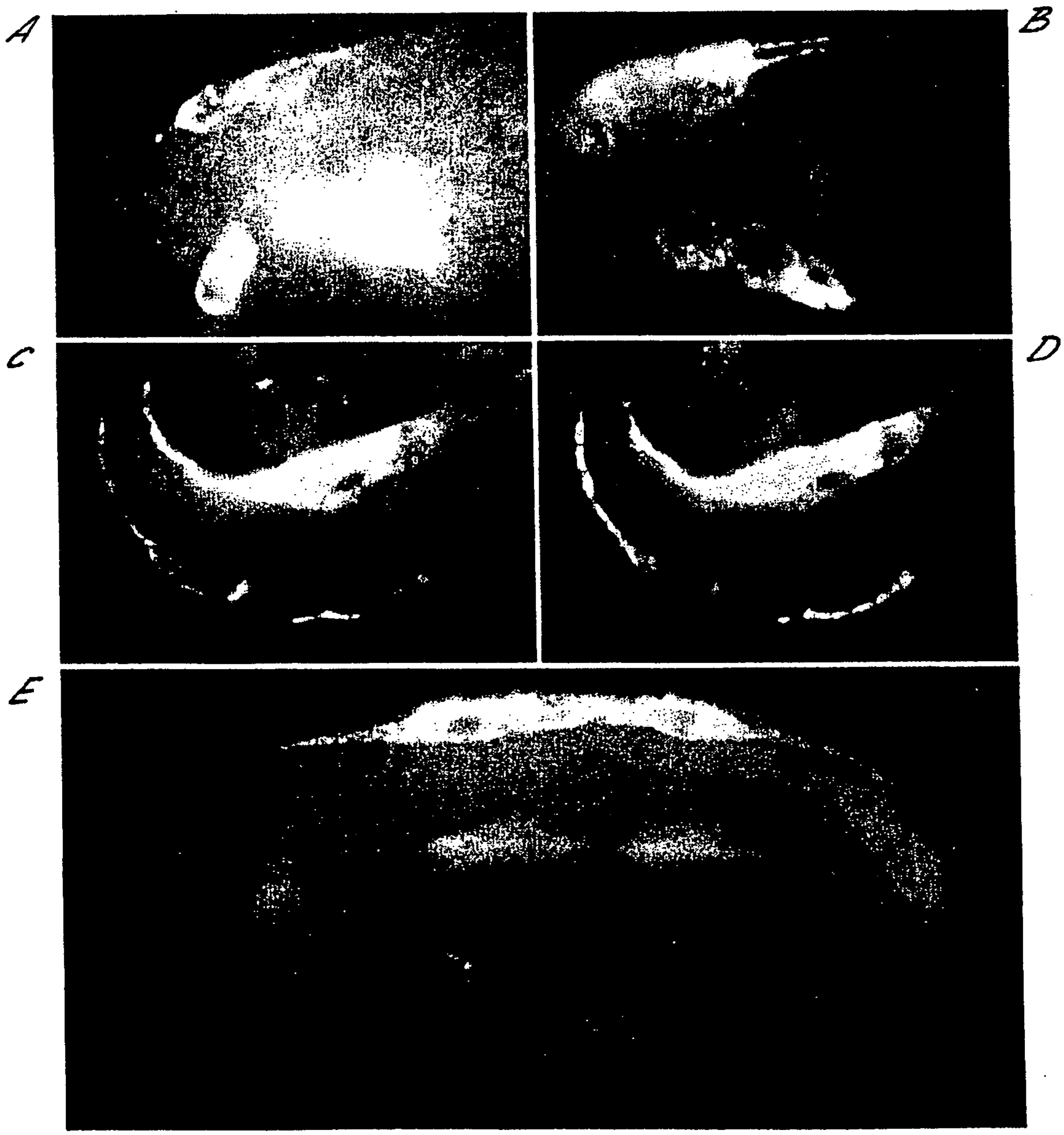

FIG. 38.

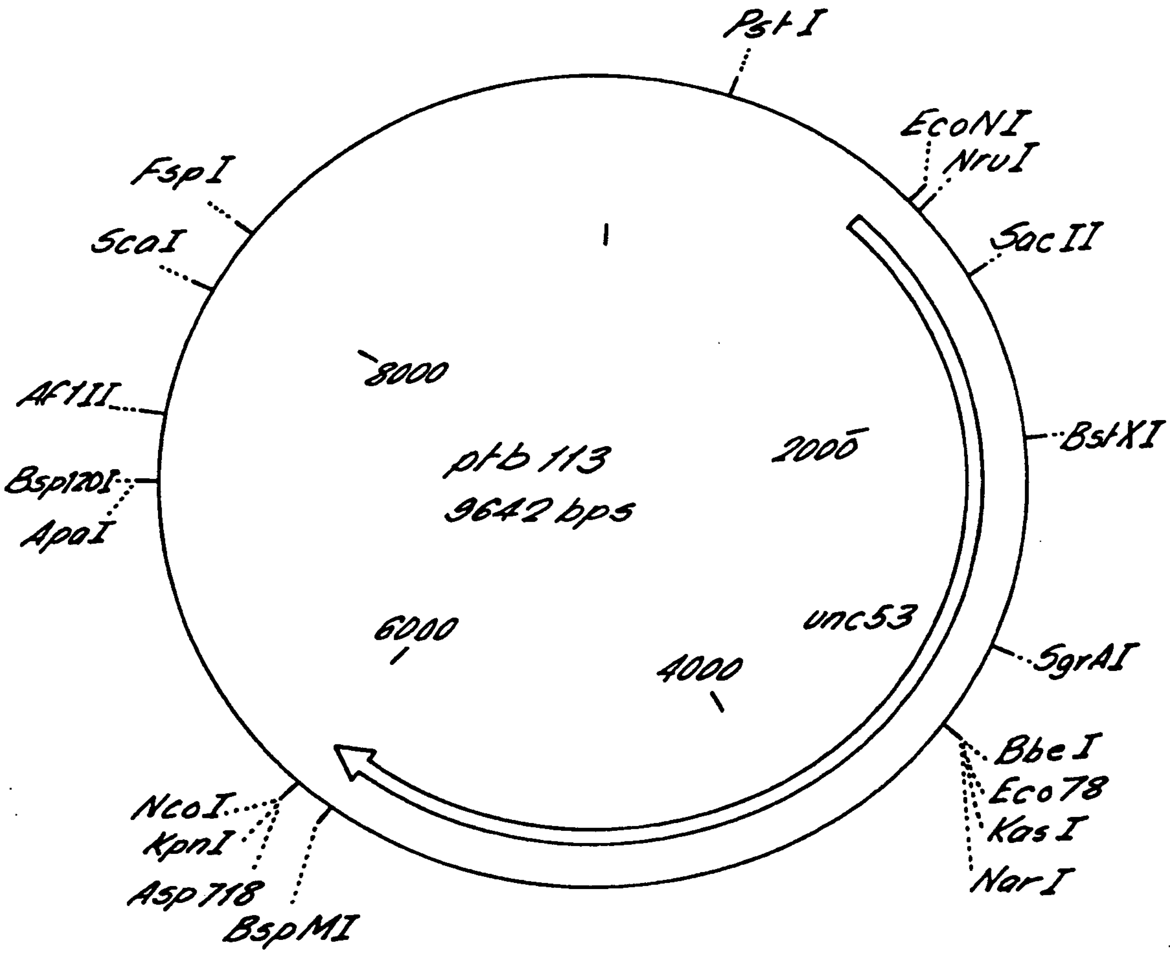
PstI
EcoNI
NruI
SacII
BstXI
SgrAI
BbeI
Eco78
KasI
NarI
BspMI
Asp718
KpnI
NcoI
ApaI
Bsp120I
AflII
ScaI
FspI
1
2000
4000
6000
8000
ptb 113
9642 bps
unc53

FIG. 39.

ATGACCATGA	TTACGCCAAG	CTTGTCTTCT	TCTAAATTCC	CATAAAATCC	CGAAACTCCT	60
TCCCTCTATC	TTCTTTTTCT	TCTCGTTTTC	AAATGTTTCT	CTCTATCCCA	TTCTCTCATC	120
AATTGAGTGG	GATGAGGCTA	TCTCTGCCTC	TCTTCTGAAT	CTCTGAACCA	TCTTACATTA	180
CACTGTGGAT	GACGAGCCCC	ACAGGCTCCC	TTGCATCAGA	TACTGCCATT	GGGGATGGCA	240
AAGAAGAGAG	AAGGTATTGT	GAGGATATAT	TTTTCTAAGA	AAAAACGTTT	GAAGAAAAGA	300
AGATGAAGAA	GATCTGCTTG	ATTCATTGCA	CAAGTTAGAA	GTAACAGGGG	TCTATATTTC	360
GAAGAACTTA	AAGGGAATGC	AACTGAACAT	AAAATTAAAC	AAAGGGATTG	AATCCTGCAG	420
TGAGTATTTT	CGGTTTTTCA	CTGGTTCTCT	GTAAAAAGAG	TAATGCAAAG	GGCAAGTTAA	480
CTTAGGTCGT	AAATGTATTG	AATTTGCTTA	AAATCTGAAG	ATCTAGTGGT	GAACCGTGGA	540
AGATTATCAA	GAGGAGGCTG	AAGATCTGTT	TAAGAACCAT	TAATCAAACT	GGTATTCTAT	600
TTTCACTGGT	TGTATGTAAA	CATTCTATCT	TATTCCTTTT	ATCACTGTTC	TGCACTTTCC	660

FIG. 39 CONTINUED. 93/99

TATAAAAAAA GTTGACCGAC CGTACTCTCT GAATTCATTT TTCCCGATCT TACCAACTCC 720
CGATCTATCT CTATCCCTGG TTTTTTCTTC GTGCTCCAAT GGAATTCTTG AGACTTCCAC 780
TATCTTCTCT GGCACCCTCC ACTACGCGTA GGCGTCTCTC GCTTCGTGTA TTCCCGGGAA 840
GCCGGTTCCC GTCTCTCCCG CCGCTGCCGC TGCCGCACAC AGCTTTACAC CTCGTAGAAT 900
CCCCAAAGAG GGGCGTGGCT TGCGGGTGCC AACATCCTCC TGCCGAGGAA GAAGCAGGCA 960
CTCATCACTC GCATCATCAA CCTCGGGATT GGCCAAAGGA CCCAAAGGTA TGTTTCGAAT 1020
GATACTAACA TAACATAGAA CATTTTCAGG AGGACCCTTG GCTAGAACTA GTGGATCCGA 1080
GCTCTCCCAT ATGACGACGT CAAATGTAGA ATTGATACCA ATCTACACGG ATTGGGCCAA 1140
TCGGCACCTT TCGAAGGGCA GCTTATCAAA GTCGATTAGG GATATTTCCA ATGATTTTCG 1200
CGACTATCGA CTGGTTTCTC AGCTTATTAA TGTGATCGTT CCGATCAACG AATTCTCGCC 1260
TGCATTCACG AAACGTTTGG CAAAAATCAC ATCGAACCTG GATGGCCTCG AAACGTGTCT 1320
CGACTACCTG AAAAATCTGG GTCTCGACTG CTCGAAACTC ACCAAAACCG ATATCGACAG 1380
CGGAAACTTG GGTGCAGTTC TCCAGCTGCT CTTCCTGCTC TCCACCTACA AGCAGAAGCT 1440
TCGGCAACTG AAAAAAGATC AGAAGAAATT GGAGCAACTA CCCACATCCA TTATGCCACC 1500
CGCGGTTTCT AAATTACCCT CGCCACGTGT CGCCACGTCA GCAACCGCTT CAGCAACTAA 1560
CCCAAATTCC AACTTTCCAC AAATGTCAAC ATCCAGGCTT CAGACTCCAC AGTCAAGAAT 1620
ATCGAAAATT GATTCATCAA AGATTGGTAT CAAGCCAAAG ACGTCTGGAC TTAAACCACC 1680
CTCATCATCA ACCACTTCAT CAAATAATAC AAATTCATTC CGTCCGTCGA GCCGTTCGAG 1740
TGGCAATAAT AATGTTGGCT CGACGATATC CACATCTGCG AAGAGCTTAG AATCATCATC 1800
AACGTACAGC TCTATTTCGA ATCTAAACCG ACCTACCTCC CAACTCCAAA AACCTTCTAG 1860
ACCACAAACC CAGCTAGTTC GTGTTGCTAC AACTACAAAA ATCGGAAGCT CAAAGCTAGC 1920
CGCTCCGAAA GCCGTGAGCA CCCCAAAACT TGCTTCTGTG AAGACTATTG GAGCAAAACA 1980
AGAGCCCGAT AACAGCGGTG GTGGTGGTGG TGGAATGCTG AAATTAAAGT TATTCAGTAG 2040
CAAAAACCCA TCTTCCTCAT CGAATAGCCC ACAACCTACG AGAAAGGCGG CGGCGGTGCC 2100
TCAACAACAA ACTTTGTCGA AAATCGCTGC CCCAGTGAAA AGTGGCCTGA AGCCGCCGAC 2160
CAGTAAGCTG GGAAGTGCCA CGTCTATGTC GAAGCTTTGT ACGCCAAAAG TTTCCTACCG 2220
TAAAACGGAC GCCCCAATCA TATCTCAACA AGACTCGAAA CGATGCTCAA AGAGCAGTGA 2280
AGAAGAGTCC GGATACGCTG GATTCAACAG CACGTCGCCA ACGTCATCAT CGACGGAAGG 2340
TTCCCTAAGC ATGCATTCCA CATCTTCCAA GAGTTCAACG TCAGACGAAA AGTCTCCGTC 2400
ATCAGACGAT CTTACTCTTA ACGCCTCCAT CGTGACAGCT ATCAGACAGC CGATAGCCGC 2460
AACACCGGTT TCTCCAAATA TTATCAACAA GCCTGTTGAG GAAAAACCAA CACTGGCAGT 2520
GAAAGGAGTG AAAAGCACAG CGAAAAAAGA TCCACCTCCA GCTGTTCCGC CACGTGACAC 2580

FIG. 39 CONTINUED. 94/99

CCAGCCAACA ATCGGAGTTG TTAGTCCAAT TATGGCACAT AAGAAGTTGA CAAATGACCC 2640
CGTGATATCT GAAAAACCAG AACCTGAAAA GCTCCAATCA ATGAGCATCG ACACGACGGA 2700
CGTTCCACCG CTTCCACCTC TAAAATCAGT TGTTCCACTT AAAATGACTT CAATCCGACA 2760
ACCACCAACG TACGATGTTC TTCTAAAACA AGGAAAAATC ACATCGCCTG TCAAGTCGTT 2820
TGGATATGAG CAGTCGTCCG CGTCTGAAGA CTCCATTGTG GCTCATGCGT CGGCTCAGGT 2880
GACTCCGCCG ACAAAAACTT CTGGTAATCA TTCGCTGGAG AGAAGGATGG GAAAGAATAA 2940
GACATCAGAA TCCAGCGGCT ACACCTCTGA CGCCGGTGTT GCGATGTGCG CCAAAATGAG 3000
GGAGAAGCTG AAAGAATACG ATGACATGAC TCGTCGAGCA CAGAACGGCT ATCCTGACAA 3060
CTTCGAAGAC AGTTCCTCCT TGTCGTCTGG AATATCCGAT AACAACGAGC TCGACGACAT 3120
ATCCACGGAC GATTTGTCCG GAGTAGACAT GGCAACAGTC GCCTCCAAAC ATAGCGACTA 3180
TTCCCACTTT GTTCGCCATC CCACGTCTTC TTCCTCAAAG CCCCGAGTCC CCAGTCGGTC 3240
CTCCACATCA GTCGATTCTC GATCTCGAGC AGAACAGGAG AATGTGTACA AACTTCTGTC 3300
CCAGTGCCGA ACGAGCCAAC GTGGCGCCGC TGCCACCTCA ACCTTCGGAC AACATTCGCT 3360
AAGATCCCCG GGATACTCAT CCTATTCTCC ACACTTATCA GTGTCAGCTG ATAAGGACAC 3420
AATGTCTATG CACTCACAGA CTAGTCGACG ACCTTCTTCA CAAAAACCAA GCTATTCAGG 3480
CCAATTTCAT TCACTTGATC GTAAATGCCA CCTTCAAGAG TTCACATCCA CCGAGCACAG 3540
AATGGCGGCT CTCTTGAGCC CGAGACGGGT GCCGAACTCG ATGTCGAAAT ATGATTCTTC 3600
AGGATCCTAC TCGGCGCGTT CCCGAGGTGG AAGCTCTACT GGTATCTATG GAGAGACGTT 3660
CCAACTGCAC AGACTATCCG ATGAAAAATC CCCCGCACAT TCTGCCAAAA GTGAGATGGG 3720
ATCCCAACTA TCACTGGCTA GCACGACAGC ATATGGATCT CTCAATGAGA AGTACGAACA 3780
TGCTATTCGG GACATGGCAC GTGACTTGGA GTGTTACAAG AACACTGTCG ACTCACTAAC 3840
CAAGAAACAG GAGAACTATG GAGCATTGTT TGATCTTTTT GAGCAAAAGC TTAGAAAACT 3900
CACTCAACAC ATTGATCGAT CCAACTTGAA GCCTGAAGAG GCAATACGAT TCAGGCAGGA 3960
CATTGCTCAT TTGAGGGATA TTAGCAATCA TCTTGCATCC AACTCAGCTC ATGCTAACGA 4020
AGGCGCTGGT GAGCTTCTTC GTCAACCATC TCTGGAATCA GTTGCATCCC ATCGATCATC 4080
GATGTCATCG TCGTCGAAAA GCAGCAAGCA GGAGAAGATC AGCTTGAGCT CGTTTGGCAA 4140
GAACAAGAAG AGCTGGATCC GCTCCTCACT CTCCAAGTTC ACCAAGAAGA AGAACAAGAA 4200
CTACGACGAA GCACATATGC CATCAATTTC CGGATCTCAA GGAACTCTTG ACAACATTGA 4260
TGTGATTGAG TTGAAGCAAG AGCTCAAAGA ACGCGATAGT GCACTTTACG AAGTCCGCCT 4320
TGACAATCTG GATCGTGCCC GCGAAGTTGA TGTTCTGAGG GAGACAGTGA ACAAGTTGAA 4380
AACCGAGAAC AAGCAATTAA AGAAAGAAGT GGACAAACTC ACCAACGGTC CAGCCACTCG 4440
TGCTTCTTCC CGCGCCTCAA TTCCAGTTAT CTACGACGAT GAGCATGTCT ATGATGCAGC 4500

SUBSTITUTE SHEET (RULE 26)

FIG. 39 CONTINUED. 95/99

GTGTAGCAGT ACATCAGCTA GTCAATCTTC GAAACGATCC TCTGGCTGCA ACTCAATCAA 4560
GGTTACTGTA AACGTGGACA TCGCTGGAGA AATCAGTTCG ATCGTTAACC CGGACAAAGA 4620
GATAATCGTA GGATATCTTG CCATGTCAAC CAGTCAGTCA TGCTGGAAAG ACATTGATGT 4680
TTCTATTCTA GGACTATTTG AAGTCTACCT ATCCAGAATT GATGTGGAGC ATCAACTTGG 4740
AATCGATGCT CGTGATTCTA TCCTTGGCTA TCAAATTGGT GAACTTCGAC GCGTCATTGG 4800
AGACTCCACA ACCATGATAA CCAGCCATCC AACTGACATT CTTACTTCCT CAACTACAAT 4860
CCGAATGTTC ATGCACGGTG CCGCACAGAG TCGCGTAGAC AGTCTGGTCC TTGATATGCT 4920
TCTTCCAAAG CAAATGATTC TCCAACTCGT CAAGTCAATT TTGACAGAGA GACGTCTGGT 4980
GTTAGCTGGA GCAACTGGAA TTGGAAAGAG CAAACTGGCG AAGACCCTGG CTGCTTATGT 5040
ATCTATTCGA ACAAATCAAT CCGAAGATAG TATTGTTAAT ATCAGCATTC CTGAAAACAA 5100
TAAAGAAGAA TTGCTTCAAG TGGAACGACG CCTGGAAAAG ATCTTGAGAA GCAAAGAATC 5160
ATGCATCGTA ATTCTAGATA ATATCCCAAA GAATCGAATT GCATTTGTTG TATCCGTTTT 5220
TGCAAATGTC CCACTTCAAA ACAACGAAGG TCCATTTGTA GTATGCACAG TCAACCGATA 5280
TCAAATCCCT GAGCTTCAAA TTCACCACAA TTTCAAAATG TCAGTAATGT CGAATCGTCT 5340
CGAAGGATTC ATCCTACGTT ACCTCCGACG ACGGGCGGTA GAGGATGAGT ATCGTCTAAC 5400
TGTACAGATG CCATCAGAGC TCTTCAAAAT CATTGACTTC TTCCCAATAG CTCTTCAGGC 5460
CGTCAATAAT TTTATTGAGA AAACGAATTC TGTTGATGTG ACAGTTGGTC CAAGAGCATG 5520
CTTGAACTGT CCTCTAACTG TCGATGGATC CCGTGAATGG TTCATTCGAT TGTGGAATGA 5580
GAACTTCATT CCATATTTGG AACGTGTTGC TAGAGATGGC AAAAAAACCT TCGGTCGCTG 5640
CACTTCCTTC GAGGATCCCA CCGACATCGT CTCTAAAAAA TGGCCGTGGT TCGATGGTGA 5700
AAACCCGGAG AATGTGCTCA AACGTCTTCA ACTCCAAGAC CTCGTCCCGT CACCTGCCAA 5760
CTCATCCCGA CAACACTTCA ATCCCCTCGA GTCGTTGATC CAATTGCATG CTACCAAGCA 5820
TCAGACCATC GACAACATTT GAACAGAAGA CTCTAATCTT CTCTCGCCTC TCCCCCGCTT 5880
TCCTTATCTT CGTACCGGTA CCATGGTATT GATATCTGAG CTCCGCATCG GCCGCTGTCA 5940
TCAGATCGCC ATCTCGCGCC CGTGCCTCTG ACTTCTAAGT CCAATTACTC TTCAACATCC 6000
CTACATGCTC TTTCTCCCTG TGCTCCCACC CCCTATTTTT GTTATTATCA AAAAAACTTC 6060
TTCTTAATTT CTTTGTTTTT TAGCTTCTTT TAAGTCACCT CTAACAATGA AATTGTGTAG 6120
ATTCAAAAAT AGAATTAATT CGTAATAAAA AGTCGAAAAA AATTGTGCTC CCTCCCCCCA 6180
TTAATAATAA TTCTATCCCA AAATCTACAC AATGTTCTGT GTACACTTCT TATGTTTTTT 6240
TTACTTCTGA TAAATTTTTT TTGAAACATC ATAGAAAAAA CCGCACACAA AATACCTTAT 6300
CATATGTTAC GTTTCAGTTT ATGACCGCAA TTTTTATTTC TTCGCACGTC TGGGCCTCTC 6360
ATGACGTCAA ATCATGCTCA TCGTGAAAAA GTTTTGGAGT ATTTTTGGAA TTTTTCAATC 6420

FIG. 39 CONTINUED. 96/99

AAGTGAAAGT TTATGAAATT AATTTTCCTG CTTTTGCTTT TTGGGGGTTT CCCCTATTGT 6480
TTGTCAAGAG TTTCGAGGAC GGCGTTTTTC TTGCTAAAAT CACAAGTATT GATGAGCACG 6540
ATGCAAGAAA GATCGGAAGA AGGTTTGGGT TTGAGGCTCA GTGGAAGGTG AGTAGAAGTT 6600
GATAATTTGA AAGTGGAGTA GTGTCTATGG GGTTTTTGCC TTAAATGACA GAATACATTC 6660
CCAATATACC AAACATAACT GTTTAAAATT AAACATTTTT CTAAATTTTA TATGATTTCT 6720
TTTAAATTTG CAAAAATTAC TTAAATTTGA ATTCCCGCGC AAATGAGTGA CTTCATTTTC 6780
TGCATTATTG TGTTTTCCGG CTATATTAAT AGGTATTTGT TTGTGTTTTT CTTTATTTTA 6840
TGATTCGAAC TCCAATTTGT AAATTTTCGA ACATATTTCC CTAAAGAAAA AATATGATTA 6900
ATCTGGAAAA ATTGGAAAAT TATTTTTCAA ATAAAAAACA AAGAAAAAAA TGAAGAAAAA 6960
CCTATTAGTT TGGCCATAAA ACGCAAAAAT GTCGAAAATG ACGTCACTCA TCTGCGCGGG 7020
AAATCAAGAA TAATTCGGCC TTTTTTATTT TTTTGGAAAA TCGTAAAACA TTTAGAAAAA 7080
TTTTTTAATA GTTATAGTGG GACTGTATTC TGTCATTTAG GGCAAAAGCC AGAGACGCTA 7140
CTCCACCGTT GGGGGATCCA CTAGTCGGCC GTACGGGCCC TTTCGTCTCG CGCGTTTCGG 7200
TGATGACGGT GAAAACCTCT GACACATGCA GCTCCCGGAG ACGGTCACAG CTTGTCTGTA 7260
AGCGGATGCC GGGAGCAGAC AAGCCCGTCA GGGCGCGTCA GCGGGTGTTG GCGGGTGTCG 7320
GGGCTGGCTT AACTATGCGG CATCAGAGCA GATTGTACTG AGAGTGCACC ATATGCGGTG 7380
TGAAATACCG CACAGATGCG TAAGGAGAAA ATACCGCATC AGGCGGCCTT AAGGGCCTCG 7440
TGATACGCCT ATTTTTATAG GTTAATGTCA TGATAATAAT GGTTTCTTAG ACGTCAGGTG 7500
GCACTTTTCG GGGAAATGTG CGCGGAACCC CTATTTGTTT ATTTTTCTAA ATACATTCAA 7560
ATATGTATCC GCTCATGAGA CAATAACCCT GATAAATGCT TCAATAATAT TGAAAAAGGA 7620
AGAGTATGAG TATTCAACAT TTCCGTGTCG CCCTTATTCC CTTTTTTGCG GCATTTTGCC 7680
TTCCTGTTTT TGCTCACCCA GAAACGCTGG TGAAAGTAAA AGATGCTGAA GATCAGTTGG 7740
GTGCACGAGT GGGTTACATC GAACTGGATC TCAACAGCGG TAAGATCCTT GAGAGTTTTC 7800
GCCCCGAAGA ACGTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT GGCGCGGTAT 7860
TATCCCGTAT TGACGCCGGG CAAGAGCAAC TCGGTCGCCG CATACACTAT TCTCAGAATG 7920
ACTTGGTTGA GTACTCACCA GTCACAGAAA AGCATCTTAC GGATGGCATG ACAGTAAGAG 7980
AATTATGCAG TGCTGCCATA ACCATGAGTG ATAACACTGC GGCCAACTTA CTTCTGACAA 8040
CGATCGGAGG ACCGAAGGAG CTAACCGCTT TTTTGCACAA CATGGGGGAT CATGTAACTC 8100
GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC AAACGACGAG CGTGACACCA 8160
CGATGCCTGT AGCAATGGCA ACAACGTTGC GCAAACTATT AACTGGCGAA CTACTTACTC 8220
TAGCTTCCCG GCAACAATTA ATAGACTGGA TGGAGGCGGA TAAAGTTGCA GGACCACTTC 8280
TGCGCTCGGC CCTTCCGGCT GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG 8340

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FIG. 39 CONTINUED.

GGTCTCGCGG TATCATTGCA GCACTGGGGC CAGATGGTAA GCCCTCCCGT ATCGTAGTTA 8400
TCTACACGAC GGGGAGTCAG GCAACTATGG ATGAACGAAA TAGACAGATC GCTGAGATAG 8460
GTGCCTCACT GATTAAGCAT TGGTAACTGT CAGACCAAGT TTACTCATAT ATACTTTAGA 8520
TTGATTTAAA ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT TTTGATAATC 8580
TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA 8640
AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA 8700
AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC 8760
CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCCTTCTA GTGTAGCCGT 8820
AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC 8880
TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC 8940
GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA 9000
GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCAT TGAGAAAGCG 9060
CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG 9120
GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT 9180
TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT 9240
GGAAAAACGC CAGCAACGCG GCCTTTTTAC GGTTCCTGGC CTTTTGCTGG CCTTTTGCTC 9300
ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT 9360
GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG 9420
CGGAAGAGCG CCCAATACGC AAACCGCCTC TCCCCGCGCG TTGGCCGATT CATTAATGCA 9480
GCTGGCACGA CAGGTTTCCC GACTGGAAAG CGGGCAGTGA GCGCAACGCA ATTAATGTGA 9540
GTTAGCTCAC TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT 9600
GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CT 9642

FIG. 40. 98/99

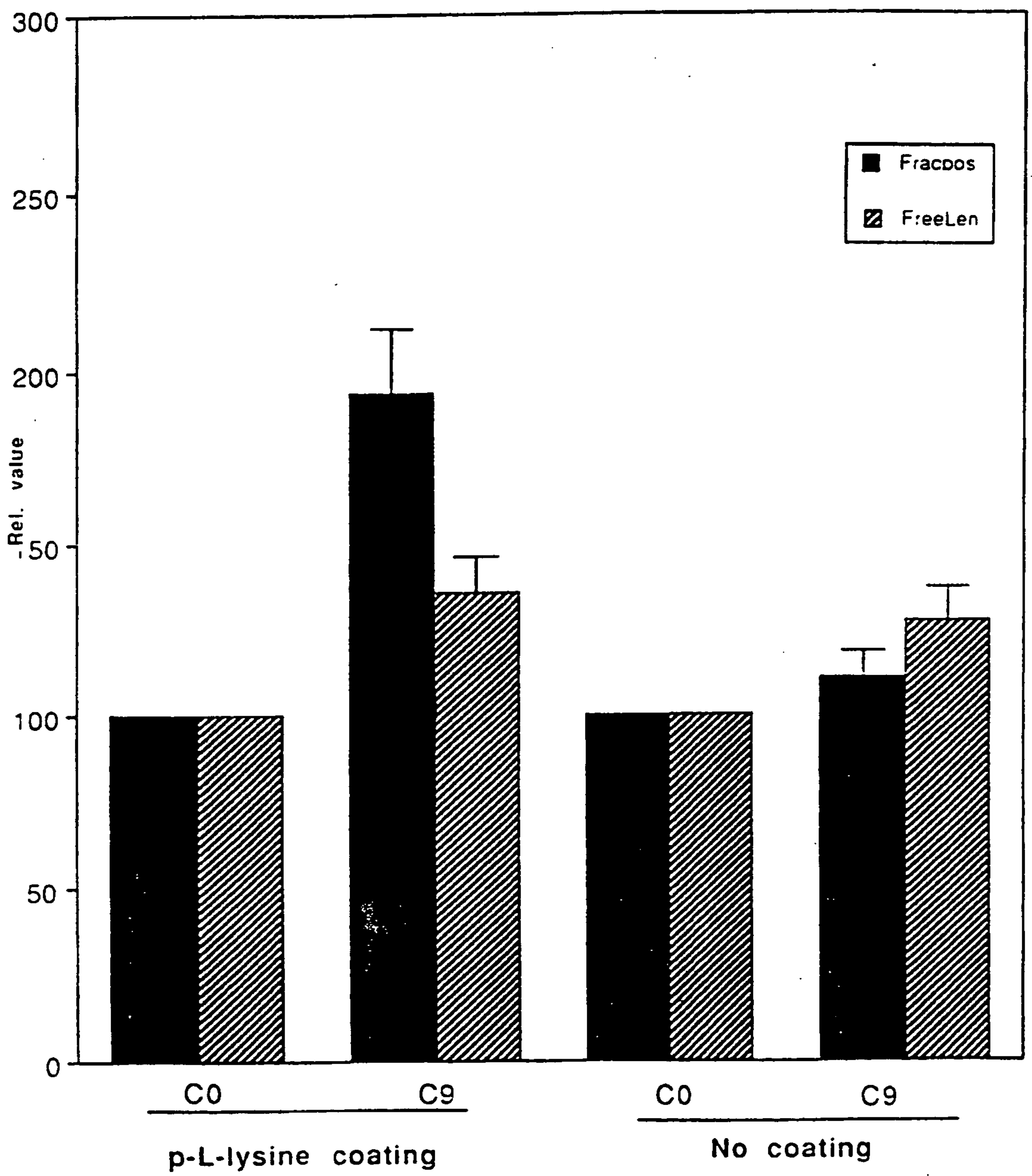
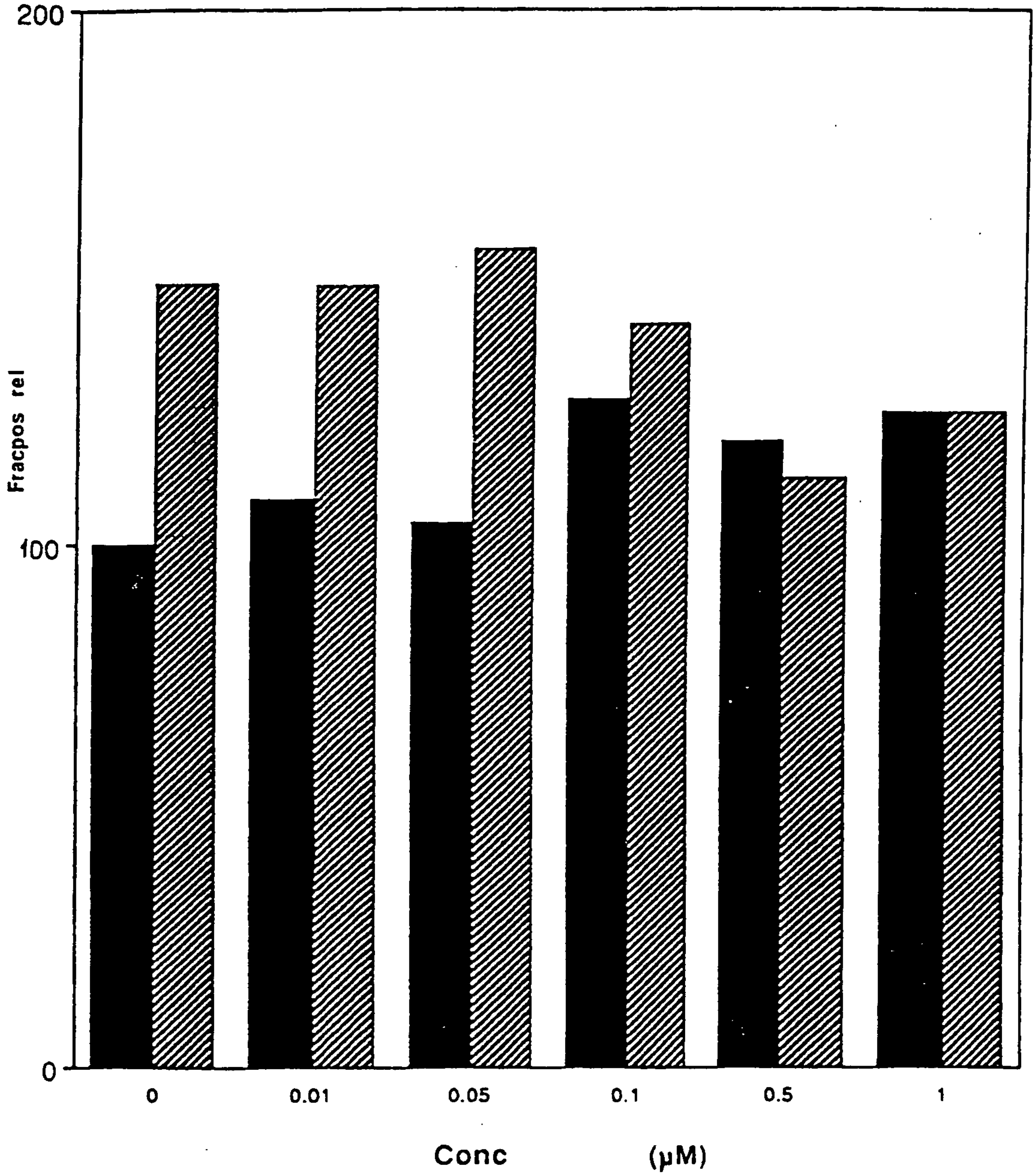

300
250
200
-Rel. value
-50
100
50
0
Fracpos
FreeLen
C0
C9
p-L-lysine coating
C0
C9
No coating

FIG. 41



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(57) Abstract

UNC-53 protein of C. elegans or its functional equivalent is identified as a signal transducer/integrator involved in controlling the rate and directionality of cell migration and/or cell shape. Nucleic acid sequences encoding UNC-53 protein or its functional equivalent, such as genomic or cDNA are used to transfect C. elegans or mammalian cell lines useful for identifying inhibitors or enhancers of the UNC-53 protein. Any of the inhibitors or enhancers identified or the UNC-53 protein itself or sequences encoding UNC-53 protein can be used in the preparation of medicament for treatment of neurological conditions such as Alzheimer's or Huntingdon's disease, peripheral neuropathies for inhibition of metastasis.

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Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	EMBL Database Entry CEF45E10 Accession number Z47810; 26 January 1995 XP002019188 & NATURE, vol. 368, no. 6466, 3 April 1994, LONDON GB, pages 32-38, R. WILSON ET AL.: "2.2 Mb of contiguous nucleotide sequence from chromosome III of C. elegans" see the whole document --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
4 December 1996	13. 12. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B

Form PCT/ISA/210 (second sheet) (July 1992)

Intc onal Application No
PCT/EP 96/02311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF NEUROSCIENCE 13 (10). 1993. 4254-4271. ISSN: 0270-6474, XP000612286 HEKIMI S ET AL: "Axonal guidance defects in a Caenorhabditis elegans mutant reveal cell-extrinsic determinants of neuronal morphology."	19,43
A	see abstract see page 4255, left-hand column, paragraph 2 - paragraph 3 see page 4267, right-hand column, paragraph 2 - page 4271, left-hand column, paragraph 3	1-18, 20-42, 44-88

4

the 'round' population as well as by driving a fraction of transfected MCF-7 cells from non-directional motility (round tracks) into directional migration (linear tracks).

In tissue culture, cells are provided with non-directional signals. It is likely that providing directionality to these signals will enhance observed effects. Significant enhancement was observed for the fraction of linear tracks.

In addition, a significant increase of 35% in the area of tracks was observed in the Ce-unc-53 transfected MCF-7 cells versus the parent MCF-7 cells (Table 3). This increase occurred in the round track population; the area of linear tracks was found not to be changed by transfection.

These obsevations in phagokinesis suggest that Ce-unc-53 transfection into MCF-7 cells is capable of increasing insitu locomotion in Ce-unc-53 MCF-7, e.g. by increasing spreading, ruffling, or other forms of non-directional motility in the "round" population. In addition the Ce-unc-53 transgene in MCF-7 cells drives a fraction of the MCF-7 cells from non-directional motility (round tracks) into directional migration (linear tracks).

Table 3. Analysis of phagokinesis assays with parent and *Ce-unc-53* transfected MCF-7 cells.

	parent MCF-7		*Ce-unc-53 MCF-7*		*Increase*
Fraction linear tracks (*)	*% +- SD(n)* 12+-3 (8)		*%+-SD(n)* 28+-6 (8)		2.33
Track area (**)	*pixels+-SD(n)*		*picels+-SD (n)*		
all tracks	1261+-128(8)		1698+-179(8)		1.35
round tracks	1229+-162(8)		1464+-204(8)		1.19
linear tracks	2367+-424(8)		2300+-319(8)		0.97

(*) the fraction of linear tracks in 8 wells was pooled.

MCF-7 cells expressing low levels of UNC-53 exhibit increased motility.

Individual transfected cells are much more flattened in appearance than wild type and have a broad lamellipodium extending from the edge of the cell. Ruffling edges are more frequent than in wild type. Transfected cells in clusters have a broad lamellipodium edge around the cluster while cluster of the non-transfected. Within the cluster the nuclei are more widely spaced from one-another than in wild type cells (also due to a lamellipodium edge).

Example 11

Method for Protein micro-sequencing of co-affinity purifying proteins

UNC-53 protein was immuno-affinity purified from extracts of cells expressing C. elegans UNC-53 using monoclonal antibody 16-48-2. One to five mg of Mab 16-48-2 was prepared, purified on protein-G sepharose and subsequently covalently linked to sepharose beads. A column of such beads was loaded with both crude cytosolic and Triton-X100 extracts (containing solubilised RTKs) and eluted with 4M $MgCl_2$ or other chaotropic agents. A co-immuno-purifying band was identified on SDS-denaturing PAGE gels, eluted from these gels and micro-sequenced. This protein sequence or mass information of peptides generated by proteolysis was used to identify the co-immunoprecipitation directly from the sequence databases.

Alternatively the sequence was reverse translated

and oligonucleotides based on the sequence prepared. This is used to clone the corresponding gene as well as other techniques well known in the art.

<u>Example 12</u> <u>C. elegans</u> as a model assay system.

We have constructed transgenic strains which overexpress UNC-53 in body muscle. This results in increased extension of muscle cells and embryonic lethality at low frequency. These strains were used to screen for drugs which interfere with UNC-53 activity and thereby suppress the background lethality.

Another related assay was used to screen specifically to identify inhibitors of downstream components in the signal transduction pathway. This assay utilised constitutively active mutant cDNA (or corresponding nucleic acid sequence). Such a mutant may be formed by mutating the nucleotide binding domain such that GTP or ATP is always bound or by covalently attaching SEM-5. In this strategy, transgenics/mutants (nematodes or tissue cultured cell lines) were generated which maintain the pathway in a permanently switched on state. Over-extension and subsequent lethality results in a greater frequency than that observed in the unc-54 - unc-53 wild-type lines. By screening for survivors after drug treatment, this assay specifically identifies inhibitors of downstream components in the signal transduction pathway.

A range of other embodiments of the assay are obvious to a person skilled in the art of <u>C. elegans</u> genetics, including the use of alternative selectable markers, genetic backgrounds, histochemical detection and visual detection systems to identify phenotypic

changes following contacting a single worm or a population of worms with a compound.

Another assay previously described herein utilizes the unc-53 promoter. The unc-53 promoter is fused to a nucleic acid sequence encoding a reporter molecule. By screening for cells which do not express the wild type pattern, molecules which increase or reduce transcription of unc-53 may be identified.

Example 13 - Heterologous expression of C. elegans UNC-53 in insect cells.

C. elegans UNC53 cDNAs have been expressed in a Baculovirus system to obtain sufficient amounts of protein for biochemical and structural studies.

Two UNC53 cDNA clones (UNC53(7A) and UNC53(8A) have been documented differing in the number of adenosine (A) residues (7 or 8) in a polyA stretch of the of the 3′ coding region; the two clones therefore have different reading frames in the carboxyterminal coding region.

The 5′ (N-terminal) part of the UNC53 coding region was excised from pTB564 with *SacII* after linearizing the plasmid with *NdeI* . The *Ndei* site was blunted with Klenow. The remaining C-terminal part of the coding region was excised from pTB68(7A) and pTB50(8A) with *SacII* plus *KpnI*. The *NdeI/SacII* fragment from pTB64 and the *SacII/KpnI* fragment from either pTB68 or pTB50 were ligated simultaneously into pBacPAK9 (Clontech) which had been linearized with *Ecl136II* (blunt end) and *KpnI*. In this way, a minimum amount of 5′ untranslated region is left in the final construct.

The desired recombinant viruses were obtained by

co-transfection of *Sf*21 cells (*Spodoptera frugiperda*) with one of the aforementioned pBacPAK9 constructs and BacPAK6 *Bsu*361-digested DNA (Clontech). Several candidate recombinant viruses plaques were picked and screened by PCR for the presence of the target gene and the absence of wild-type virus.

*Sf*9 cells were infected at a high multiplicity with UNC53(7A) or UNC53(8A) recombinant Baculoviruses for protein expression. Proteins from whole cell lysates were separated by denaturing (SDS) polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The expression of UNC53 in those cell lysates was confirmed by immunoreaction with a monoclonal antibody (16-49-2) to UNC53 and subsequent chemiluminescent detection (ECL™ Amersham). A Coomassie-stained band of the expected size was observed in lysates of *Sf*9 cells infected with UNC-53(7A) or UNC53(8A) recombinant baculoviruses, but not with control constructs. Within the accuracy of the methods, this Coomassie-stained band coincided with the largest immunoreactive band. Their estimated mass was approximately 180 kDa, which is compatible with the theoretically calculated mass (167 kDa). We therefore conclude that this band most likely corresponds to intact UNC53.

For both UNC53(7A) and UNC53(8A) baculoviral expression constructs, mostly intact recombinant UNC53-protein was detected by immunoblotting in lysates from infected cells harvested 24 hours post infection. Larger amounts of recombinant protein could be detected in lysates from cells prepared during later stages of infection (48 and 72 hours post infection) but in those preparations a considerable amount of smaller fragments (presumptive degradation products) is observed.

Example 14

The UNC-53 protein expressed in Sf9 cells using a Baculovirus expression system is a valid tool to study its biochemical functions and a valid tool to identify interacting proteins.

3x10+6 SF9 cells infected with recombinant virus UNC53 7A(L2.3)/pBacPAK9 were resuspended in 100 microliter Phosphate Buffered Saline supplemented with 0.14 micromolar of pepstatin, 10 mM of benzamidine and 0.015 micromolar aprotinin. The cells were briefly sonicated and the obtained material was centrifuged at 30,000 g for 30 minutes at 4 degrees centrigade. The clear supernatant (soluble fraction) was frozen in 50% glycerol. An aliquot of this fraction was incubated in the cold room for 48 hrs. The protein samples were analyzed by SDS-PAGE, blotted to nitrocellulose and probed with mab 16-48-2. This showed that UNC-53 protein made in SF9 cells is soluble and stable under the conditions tested.

20 microlitres of the UNC-53 SF9 lysate were incubated with 5 microlitre GST-Sepharose beads loaded with equal amouts (approx. 10 microgram) of GST-GRB-2 or GST alone. The beads were rinsed 3 times in 500 microlitres of solution PBS-0.2% Tween 20 and eluted with 50 microliter SDS sample buffer. The eluted material was analyzed by SDS-PAGE and Western blot analysis with mab 16-48-2. UNC-53 was retained on the GST-GRB2 column and not on the GST demonstrating that UNC-53 interacts *in vitro* with GRB-2.

Example 15

Identification of proteins interacting with UNC-53 :

Vectors pCB50 and pCB51 were constructed as bait vectors for the yeast two hybrid system expressing resp. the full length and the carboxyterminal part of UNC-53.

pCB50 was constructed by cloning the full length UNC-53 cDNA (7A variant; *Nde*I-*Nco*I fragment from pTB74) into pAS1-CYH2 vector from Clontech. (Figure 30).

pCB51 (Figure 32) was constructed by cloning the 1880 bp *Nde*I-*Nco*I fragment from pTB74 into vector pAS1-CYH2 from Clontech. This protein encodes among others, the GTP/ATP binding domains, a leucine zipper domain, and an additional coiled-coil domain.

pCB50 and pCB51 were transformed in yeast strain Hf7C (YRG2). Expression was confirmed by western blotting using antibodies to the GAL4 protein fused to UNC-53 in these constructs. Bands of expected size (190 kd for pCB50 and 90 kd for pcB51) were observed both in yeast strains with pCB50 and pCB51 indicating that both fusion proteins are expressed in the yeast. The expression of the pCB50 and pCB51 fusion proteins in yeast strain Hf7C does not lead to expression of the LacZ or HIS reporter genes. These experiments demonstrate that the constructed fusions are useful baits in yeast two hybrid screens.

Vector pCB55 was made by cloning the 984 bp *BamH*I-*Bgl*II of pTB74 construct into the yeast two

hybrid activation vector (pGAD-424 vector from Clontech) (Figure 34). In order to check the possible interactions of UNC-53 either with itself (homodimerization) or other proteins.

This vector expresses a Gal-4 activation domain fused to amongst others the predicted coiled coil or leucine zipper domain of UNC-53.

The following combinations of plasmids were co-transformed in yeast strain HF7C : (1) pCB51 and pCB55 (2) pCB55 with control plasmid- pTD1 and (3) positive control plasmids pTD1 and PVA3 (two proteins known to interact (Bartel,P.L et al., Biotechniques Vol. 14 nr.6 (1993)). Yeast cotransformed with combination (1) and (3) grew well on -LEU;-TRYP plates and -LEU;-TRYP;-HIS plates indicating that an interacting protein is present in both co-transformations. Only yeast co-transformed with (3) was positive in a lacZ assay indicating that the observed interaction in (1) (between pCB50 and pCB 55) is weak.For co-transformation (2), colonies grew on -LEU;-TRYP plates and as expected not on -LEU;-TRYP;-HIS plates. The positive control were thus positive whereas the negative controls were negative. We conclude that there is a weak but significant interaction between pCB51 and pCB55, which is strong enough to activate the HIS but not the lacZ reporter gene in this Hf7c strain.

Example 16

Protocol to screen for components which inhibit or enhance UNC-53 using C. elegans cell line pTBIn76

Embryos from large liquid C. elegans cultures of line pTBIn76 (table 1) are collected by sucrose flotation of a bleached population (Goh and Bogaert (1991), Dev. Biol. 56, 110-156). Embryos are dispensed in 96 well microtiter plates with M9 medium and various concentrations of the compound to be tested. The embryos are allowed to hatch and are synchronised in the L1 stage by starvation. After a suitable exposure to the compound (by standard calibration) a standard quantity of E. coli (food) is dispersed in the 96 well plates, which starts C. elegans post-embryonic development. The microtiter plates are then placed in an incubator to induce heat shock and subsequently placed at 25°C to permit continued development. After 0 to 1 generations of C. elegans development wells are inspected to assess the degree of population growth inhibition. This inspection can consist of an optical density measurement to assess the amount of food consumed by the developing nematodes. Very little food is consumed when no test compound is present: most food is consumed if an UNC-53 inhibitor has blocked the lethal or subviable phenotype induced by the transgene. The inspection can also be a visual inspection of the number of healthy or subviable worms or a histochemical measurement of C. elegans viability or of the remainder of E. coli (food).

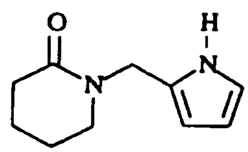
Example 17 - Protocol to screen for compounds which inhibit or enhance cell regulation or motility.

Transfected cells used in this example were the same as those obtained from example 8. Compounds to be tested were added to each of the cells and their effects on the cells monitored. Functional assays to determine neurite extension were also the same as used

in example 8 as described by Geests *et al*. One compound (of the Formula I below) was used for further testing.

Example 18 - Compounds targetted at the unc-53 pathway.

Snythesis of (1-(1H-pyrrol-2-ylmethyl)-2-piperidone.



Step 1

To a stirred solution of 150g of 1H-pyrrol-2-carboxaldehyde in 1500g parts of trichloromethane were added 690, of 5Å molecular Sieves. A kit solution of 264, of methyl 5-aminopentanoate hydrochloride in 1500g of tricholoromethane was added. After stirring for 5 minutes, 465g of thiethylamine were added over 10 minutes. Upon complete addition, the reaction mixture was stirred for 20 hours at ambient temperature. The mixture was filtered over diatomaceous earth and the filtrate was concentrated by evaporation of the solvent. The concentrate was triturated in 1,1′-oxybisethane. The precipitate was filtered off and the filtrate was concentrated, yielding 300g (91.1%) of 5-[[(1H-pyrrol-2-

Step 2

A mixture of 150g of 5-[[(1H-pyrrol-2-yl)methylen]amino]pentanoate hydrogenated at 3.10^5Pa and at ambient temperature with 3.3 parts of platinum oxide. After the calculated amount of hydrogen was consumed, the catalyst was filtered off and the filtrate was evaporated. The residue was dissolved in dichloromethane and the organic phase was washed three times with a sodium hydroxide 3 N solution. The product was distilled at 13.30 Pa (bp 100-130ºC). The residue was crystallized from cyclohexane and hexane. The product was filtered off and dried, yielding 193 parts (100%) of 1-(1H-pyrrol-2-ylmethyl)-2-piperidone. ; mp. 105.8ºC.

The compound (1-(1H-pyrrol-2-ylmethyl)-2-piperidinone) when applied for 24 hours to cultures of both wild-type and transfected N4 (mouse neuroblastoma) cells displays a differential behaviour. There is no effect (or at most a small stimulatory) effect on the wild-type N4 cells, up to concentrations of 1 μM, the compound clearly becomes toxic for both types of cells. The results indicate that this compound conteracts the effects of overexpression of UNC-53 and may have beneficial effects therefore in for example metastasis.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: BOGAERT; THIERRY
(B) STREET: Voorstraat 36 bus 11
(C) CITY: Kortrijk
(E) COUNTRY: Belgium
(F) POSTAL CODE (ZIP): B-8500

(A) NAME: STRINGHAM; EVE
(B) STREET: 9326-133 A Street
(C) CITY: Surrey
(D) STATE: British Columbia
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): V3V 5R5

(A) NAME: VANDEKERCKHOVE; JOEL
(B) STREET: Rode Benkendreef 27
(C) CITY: Loppem
(D) STATE: -
(E) COUNTRY: Belgium
(F) POSTAL CODE (ZIP): none

(ii) TITLE OF INVENTION: Processes for the identification of compounds which control cell behaviour, the compounds identified and pharmaceutical compositions containing them and their use in the control of cell behaviour

(iii) NUMBER OF SEQUENCES: 48

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP PCT/EP96/02311
(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9510944.3
(B) FILING DATE: 31-MAY-1995

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5073 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Caenorhabditis elegans

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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CGACTGGTTT CTCAGCTTAT TAATGTGATC GTTCCGATCA ACGAATTCTC GCCTGCATTC 240
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TTCATGCACG GTGCCGCACA GAGTCGCGTA GACAGTCTGG TCCTTGATAT GCTTCTTCCA 3900

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GTACTTAAAA AAAAAAAAAA AAAAAAAAAA AAA 5073

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5072 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGTTTAATTA CCCAAGTTTG AGACATCAAT TCCATCGAAC GAAATGTTGG TGCTCCGAAT 60

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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1528 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp Ala
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Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg Asp Ile
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Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln Leu Ile Asn Val
35 40 45

Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr Lys Arg Leu Ala
50 55 60

Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr Cys Leu Asp Tyr Leu
65 70 75 80

Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu Thr Lys Thr Asp Ile Asp
85 90 95

Ser Gly Asn Leu Gly Ala Val Leu Gln Leu Leu Phe Leu Leu Ser Thr
100 105 110

Tyr Lys Gln Lys Leu Arg Gln Leu Lys Lys Asp Gln Lys Lys Leu Glu
115 120 125

Gln Leu Pro Thr Ser Ile Met Pro Pro Ala Val Ser Lys Leu Pro Ser
130 135 140

Pro Arg Val Ala Thr Ser Ala Thr Ala Ser Ala Thr Asn Pro Asn Ser
145 150 155 160

Asn Phe Pro Gln Met Ser Thr Ser Arg Leu Gln Thr Pro Gln Ser Arg
165 170 175

Ile Ser Lys Ile Asp Ser Ser Lys Ile Gly Ile Lys Pro Lys Thr Ser
180 185 190

Gly Leu Lys Pro Pro Ser Ser Ser Thr Thr Ser Ser Asn Asn Thr Asn
195 200 205

Ser Phe Arg Pro Ser Ser Arg Ser Ser Gly Asn Asn Asn Val Gly Ser
210 215 220

Thr Ile Ser Thr Ser Ala Lys Ser Leu Glu Ser Ser Ser Thr Tyr Ser
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Ser Ile Ser Asn Leu Asn Arg Pro Thr Ser Gln Leu Gln Lys Pro Ser
245 250 255

Arg Pro Gln Thr Gln Leu Val Arg Val Ala Thr Thr Thr Lys Ile Gly
260 265 270

Ser Ser Lys Leu Ala Ala Pro Lys Ala Val Ser Thr Pro Lys Leu Ala
275 280 285

Ser Val Lys Thr Ile Gly Ala Lys Gln Glu Pro Asp Asn Ser Gly Gly
290 295 300

Gly Gly Gly Gly Met Leu Lys Leu Lys Leu Phe Ser Ser Lys Asn Pro
305 310 315 320

Ser Ser Ser Ser Asn Ser Pro Gln Pro Thr Arg Lys Ala Ala Ala Val
325 330 335

Pro Gln Gln Gln Thr Leu Ser Lys Ile Ala Ala Pro Val Lys Ser Gly
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Leu Lys Pro Pro Thr Ser Lys Leu Gly Ser Ala Thr Ser Met Ser Lys
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Leu Cys Thr Pro Lys Val Ser Tyr Arg Lys Thr Asp Ala Pro Ile Ile
370 375 380

Ser Gln Gln Asp Ser Lys Arg Cys Ser Lys Ser Ser Glu Glu Glu Ser
385 390 395 400

Gly Tyr Ala Gly Phe Asn Ser Thr Ser Pro Thr Ser Ser Ser Thr Glu
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Gly Ser Leu Ser Met His Ser Thr Ser Ser Lys Ser Ser Thr Ser Asp
420 425 430

Glu Lys Ser Pro Ser Ser Asp Asp Leu Thr Leu Asn Ala Ser Ile Val
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Thr Ala Ile Arg Gln Pro Ile Ala Ala Thr Pro Val Ser Pro Asn Ile
450 455 460

Ile Asn Lys Pro Val Glu Glu Lys Pro Thr Leu Ala Val Lys Gly Val
465 470 475 480

Lys Ser Thr Ala Lys Lys Asp Pro Pro Pro Ala Val Pro Pro Arg Asp
485 490 495

Thr Gln Pro Thr Ile Gly Val Val Ser Pro Ile Met Ala His Lys Lys
500 505 510

Leu Thr Asn Asp Pro Val Ile Ser Glu Lys Pro Glu Pro Glu Lys Leu
515 520 525

Gln Ser Met Ser Ile Asp Thr Thr Asp Val Pro Pro Leu Pro Pro Leu
530 535 540

Lys Ser Val Val Pro Leu Lys Met Thr Ser Ile Arg Gln Pro Pro Thr
545 550 555 560

Tyr Asp Val Leu Leu Lys Gln Gly Lys Ile Thr Ser Pro Val Lys Ser
565 570 575

Phe Gly Tyr Glu Gln Ser Ser Ala Ser Glu Asp Ser Ile Val Ala His
580 585 590

Ala Ser Ala Gln Val Thr Pro Pro Thr Lys Thr Ser Gly Asn His Ser
595 600 605

Leu Glu Arg Arg Met Gly Lys Asn Lys Thr Ser Glu Ser Ser Gly Tyr
610 615 620

Thr Ser Asp Ala Gly Val Ala Met Cys Ala Lys Met Arg Glu Lys Leu
625 630 635 640

Lys Glu Tyr Asp Asp Met Thr Arg Arg Ala Gln Asn Gly Tyr Pro Asp
645 650 655

Asn Phe Glu Asp Ser Ser Ser Leu Ser Ser Gly Ile Ser Asp Asn Asn
660 665 670

Glu Leu Asp Asp Ile Ser Thr Asp Asp Leu Ser Gly Val Asp Met Ala
675 680 685

Thr Val Ala Ser Lys His Ser Asp Tyr Ser His Phe Val Arg His Pro
690 695 700

Thr Ser Ser Ser Ser Lys Pro Arg Val Pro Ser Arg Ser Ser Thr Ser
705 710 715 720

Val Asp Ser Arg Ser Arg Ala Glu Gln Glu Asn Val Tyr Lys Leu Leu
725 730 735

Ser Gln Cys Arg Thr Ser Gln Arg Gly Ala Ala Ala Thr Ser Thr Phe
740 745 750

Gly Gln His Ser Leu Arg Ser Pro Gly Tyr Ser Ser Tyr Ser Pro His
755 760 765

Leu Ser Val Ser Ala Asp Lys Asp Thr Met Ser Met His Ser Gln Thr
770 775 780

Ser Arg Arg Pro Ser Ser Gln Lys Pro Ser Tyr Ser Gly Gln Phe His
785 790 795 800

Ser Leu Asp Arg Lys Cys His Leu Gln Glu Phe Thr Ser Thr Glu His
805 810 815

Arg Met Ala Ala Leu Leu Ser Pro Arg Arg Val Pro Asn Ser Met Ser
820 825 830

Lys Tyr Asp Ser Ser Gly Ser Tyr Ser Ala Arg Ser Arg Gly Gly Ser
835 840 845

Ser Thr Gly Ile Tyr Gly Glu Thr Phe Gln Leu His Arg Leu Ser Asp
850 855 860

Glu Lys Ser Pro Ala His Ser Ala Lys Ser Glu Met Gly Ser Gln Leu
865 870 875 880

Ser Leu Ala Ser Thr Thr Ala Tyr Gly Ser Leu Asn Glu Lys Tyr Glu
885 890 895

His Ala Ile Arg Asp Met Ala Arg Asp Leu Glu Cys Tyr Lys Asn Thr
900 905 910

Val Asp Ser Leu Thr Lys Lys Gln Glu Asn Tyr Gly Ala Leu Phe Asp
915 920 925

Leu Phe Glu Gln Lys Leu Arg Lys Leu Thr Gln His Ile Asp Arg Ser
930 935 940

Asn Leu Lys Pro Glu Glu Ala Ile Arg Phe Arg Gln Asp Ile Ala His
945 950 955 960

Leu Arg Asp Ile Ser Asn His Leu Ala Ser Asn Ser Ala His Ala Asn
965 970 975

Glu Gly Ala Gly Glu Leu Leu Arg Gln Pro Ser Leu Glu Ser Val Ala
980 985 990

Ser His Arg Ser Ser Met Ser Ser Ser Ser Lys Ser Ser Lys Gln Glu
995 1000 1005

Lys Ile Ser Leu Ser Ser Phe Gly Lys Asn Lys Lys Ser Trp Ile Arg
1010 1015 1020

Ser Ser Leu Ser Lys Phe Thr Lys Lys Lys Asn Lys Asn Tyr Asp Glu
1025 1030 1035 1040

Ala His Met Pro Ser Ile Ser Gly Ser Gln Gly Thr Leu Asp Asn Ile
1045 1050 1055

Asp Val Ile Glu Leu Lys Gln Glu Leu Lys Glu Arg Asp Ser Ala Leu
1060 1065 1070

Tyr Glu Val Arg Leu Asp Asn Leu Asp Arg Ala Arg Glu Val Asp Val
1075 1080 1085

Leu Arg Glu Thr Val Asn Lys Leu Lys Thr Glu Asn Lys Gln Leu Lys
1090 1095 1100

Lys Glu Val Asp Lys Leu Thr Asn Gly Pro Ala Thr Arg Ala Ser Ser
1105 1110 1115 1120

Arg Ala Ser Ile Pro Val Ile Tyr Asp Asp Glu His Val Tyr Asp Ala
1125 1130 1135

Ala Cys Ser Ser Thr Ser Ala Ser Gln Ser Ser Lys Arg Ser Ser Gly
1140 1145 1150

Cys Asn Ser Ile Lys Val Thr Val Asn Val Asp Ile Ala Gly Glu Ile
1155 1160 1165

Ser Ser Ile Val Asn Pro Asp Lys Glu Ile Ile Val Gly Tyr Leu Ala
1170 1175 1180

Met Ser Thr Ser Gln Ser Cys Trp Lys Asp Ile Asp Val Ser Ile Leu
1185 1190 1195 1200

Gly Leu Phe Glu Val Tyr Leu Ser Arg Ile Asp Val Glu His Gln Leu
1205 1210 1215

Gly Ile Asp Ala Arg Asp Ser Ile Leu Gly Tyr Gln Ile Gly Glu Leu
1220 1225 1230

Arg Arg Val Ile Gly Asp Ser Thr Thr Met Ile Thr Ser His Pro Thr
1235 1240 1245

Asp Ile Leu Thr Ser Ser Thr Thr Ile Arg Met Phe Met His Gly Ala
1250 1255 1260

Ala Gln Ser Arg Val Asp Ser Leu Val Leu Asp Met Leu Leu Pro Lys
1265 1270 1275 1280

Gln Met Ile Leu Gln Leu Val Lys Ser Ile Leu Thr Glu Arg Arg Leu
1285 1290 1295

Val Leu Ala Gly Ala Thr Gly Ile Gly Lys Ser Lys Leu Ala Lys Thr
1300 1305 1310

Leu Ala Ala Tyr Val Ser Ile Arg Thr Asn Gln Ser Glu Asp Ser Ile
1315 1320 1325

Val Asn Ile Ser Ile Pro Glu Asn Asn Lys Glu Glu Leu Leu Gln Val
1330 1335 1340

Glu Arg Arg Leu Glu Lys Ile Leu Arg Ser Lys Glu Ser Cys Ile Val
1345 1350 1355 1360

Ile Leu Asp Asn Ile Pro Lys Asn Arg Ile Ala Phe Val Val Ser Val
1365 1370 1375

Phe Ala Asn Val Pro Leu Gln Asn Asn Glu Gly Pro Phe Val Val Cys
1380 1385 1390

Thr Val Asn Arg Tyr Gln Ile Pro Glu Leu Gln Ile His His Asn Phe
1395 1400 1405

Lys Met Ser Val Met Ser Asn Arg Leu Glu Gly Phe Ile Leu Arg Tyr
1410 1415 1420

Leu Arg Arg Arg Ala Val Glu Asp Glu Tyr Arg Leu Thr Val Gln Met
1425 1430 1435 1440

Pro Ser Glu Leu Phe Lys Ile Ile Asp Phe Phe Pro Ile Ala Leu Gln
1445 1450 1455

Ala Val Asn Asn Phe Ile Glu Lys Thr Asn Ser Val Asp Val Thr Val
1460 1465 1470

Gly Pro Arg Ala Cys Leu Asn Cys Pro Leu Thr Val Asp Gly Ser Arg
1475 1480 1485

Glu Trp Phe Ile Arg Leu Trp Asn Glu Asn Phe Ile Pro Tyr Leu Glu
1490 1495 1500

Arg Val Ala Arg Asp Gly Lys Lys Asn Leu Arg Ser Leu His Phe Leu
1505 1510 1515 1520

Arg Gly Ser His Arg His Arg Leu
1525

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1583 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp Ala
1 5 10 15

Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg Asp Ile
20 25 30

Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln Leu Ile Asn Val
35 40 45

Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr Lys Arg Leu Ala
50 55 60

Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr Cys Leu Asp Tyr Leu
65 70 75 80

Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu Thr Lys Thr Asp Ile Asp
85 90 95

Ser Gly Asn Leu Gly Ala Val Leu Gln Leu Leu Phe Leu Leu Ser Thr
100 105 110

Tyr Lys Gln Lys Leu Arg Gln Leu Lys Lys Asp Gln Lys Lys Leu Glu
115 120 125

Gln Leu Pro Thr Ser Ile Met Pro Pro Ala Val Ser Lys Leu Pro Ser
130 135 140

Pro Arg Val Ala Thr Ser Ala Thr Ala Ser Ala Thr Asn Pro Asn Ser
145 150 155 160

Asn Phe Pro Gln Met Ser Thr Ser Arg Leu Gln Thr Pro Gln Ser Arg
165 170 175

Ile Ser Lys Ile Asp Ser Ser Lys Ile Gly Ile Lys Pro Lys Thr Ser
180 185 190

Gly Leu Lys Pro Pro Ser Ser Ser Thr Thr Ser Ser Asn Asn Thr Asn
195 200 205

Ser Phe Arg Pro Ser Ser Arg Ser Ser Gly Asn Asn Asn Val Gly Ser
210 215 220

Thr Ile Ser Thr Ser Ala Lys Ser Leu Glu Ser Ser Ser Thr Tyr Ser
225 230 235 240

Ser Ile Ser Asn Leu Asn Arg Pro Thr Ser Gln Leu Gln Lys Pro Ser
245 250 255

Arg Pro Gln Thr Gln Leu Val Arg Val Ala Thr Thr Thr Lys Ile Gly
260 265 270

Ser Ser Lys Leu Ala Ala Pro Lys Ala Val Ser Thr Pro Lys Leu Ala
275 280 285

Ser Val Lys Thr Ile Gly Ala Lys Gln Glu Pro Asp Asn Ser Gly Gly
290 295 300

Gly Gly Gly Gly Met Leu Lys Leu Lys Leu Phe Ser Ser Lys Asn Pro
305 310 315 320

Ser Ser Ser Ser Asn Ser Pro Gln Pro Thr Arg Lys Ala Ala Ala Val
325 330 335

Pro Gln Gln Gln Thr Leu Ser Lys Ile Ala Ala Pro Val Lys Ser Gly
340 345 350

Leu Lys Pro Pro Thr Ser Lys Leu Gly Ser Ala Thr Ser Met Ser Lys
355 360 365

Leu Cys Thr Pro Lys Val Ser Tyr Arg Lys Thr Asp Ala Pro Ile Ile
370 375 380

Ser Gln Gln Asp Ser Lys Arg Cys Ser Lys Ser Ser Glu Glu Glu Ser
385 390 395 400

Gly Tyr Ala Gly Phe Asn Ser Thr Ser Pro Thr Ser Ser Ser Thr Glu
405 410 415

Gly Ser Leu Ser Met His Ser Thr Ser Ser Lys Ser Ser Thr Ser Asp
420 425 430

Glu Lys Ser Pro Ser Ser Asp Asp Leu Thr Leu Asn Ala Ser Ile Val
435 440 445

Thr Ala Ile Arg Gln Pro Ile Ala Ala Thr Pro Val Ser Pro Asn Ile
450 455 460

Ile Asn Lys Pro Val Glu Glu Lys Pro Thr Leu Ala Val Lys Gly Val
465 470 475 480

Lys Ser Thr Ala Lys Lys Asp Pro Pro Pro Ala Val Pro Pro Arg Asp
485 490 495

Thr Gln Pro Thr Ile Gly Val Val Ser Pro Ile Met Ala His Lys Lys
500 505 510

Leu Thr Asn Asp Pro Val Ile Ser Glu Lys Pro Glu Pro Glu Lys Leu
515 520 525

Gln Ser Met Ser Ile Asp Thr Thr Asp Val Pro Pro Leu Pro Pro Leu
530 535 540

Lys Ser Val Val Pro Leu Lys Met Thr Ser Ile Arg Gln Pro Pro Thr
545 550 555 560

Tyr Asp Val Leu Leu Lys Gln Gly Lys Ile Thr Ser Pro Val Lys Ser
565 570 575

Phe Gly Tyr Glu Gln Ser Ser Ala Ser Glu Asp Ser Ile Val Ala His
580 585 590

Ala Ser Ala Gln Val Thr Pro Pro Thr Lys Thr Ser Gly Asn His Ser
595 600 605

Leu Glu Arg Arg Met Gly Lys Asn Lys Thr Ser Glu Ser Ser Gly Tyr
610 615 620

Thr Ser Asp Ala Gly Val Ala Met Cys Ala Lys Met Arg Glu Lys Leu
625 630 635 640

Lys Glu Tyr Asp Asp Met Thr Arg Arg Ala Gln Asn Gly Tyr Pro Asp
645 650 655

Asn Phe Glu Asp Ser Ser Ser Leu Ser Ser Gly Ile Ser Asp Asn Asn
660 665 670

Glu Leu Asp Asp Ile Ser Thr Asp Asp Leu Ser Gly Val Asp Met Ala
675 680 685

Thr Val Ala Ser Lys His Ser Asp Tyr Ser His Phe Val Arg His Pro
690 695 700

Thr Ser Ser Ser Ser Lys Pro Arg Val Pro Ser Arg Ser Ser Thr Ser
705 710 715 720

Val Asp Ser Arg Ser Arg Ala Glu Gln Glu Asn Val Tyr Lys Leu Leu
725 730 735

Ser Gln Cys Arg Thr Ser Gln Arg Gly Ala Ala Ala Thr Ser Thr Phe
740 745 750

Gly Gln His Ser Leu Arg Ser Pro Gly Tyr Ser Ser Tyr Ser Pro His
755 760 765

Leu Ser Val Ser Ala Asp Lys Asp Thr Met Ser Met His Ser Gln Thr
770 775 780

Ser Arg Arg Pro Ser Ser Gln Lys Pro Ser Tyr Ser Gly Gln Phe His
785 790 795 800

Ser Leu Asp Arg Lys Cys His Leu Gln Glu Phe Thr Ser Thr Glu His
805 810 815

Arg Met Ala Ala Leu Leu Ser Pro Arg Arg Val Pro Asn Ser Met Ser
820 825 830

Lys Tyr Asp Ser Ser Gly Ser Tyr Ser Ala Arg Ser Arg Gly Gly Ser
835 840 845

Ser Thr Gly Ile Tyr Gly Glu Thr Phe Gln Leu His Arg Leu Ser Asp
850 855 860

Glu Lys Ser Pro Ala His Ser Ala Lys Ser Glu Met Gly Ser Gln Leu
865 870 875 880

Ser Leu Ala Ser Thr Thr Ala Tyr Gly Ser Leu Asn Glu Lys Tyr Glu
885 890 895

His Ala Ile Arg Asp Met Ala Arg Asp Leu Glu Cys Tyr Lys Asn Thr
900 905 910

Val Asp Ser Leu Thr Lys Lys Gln Glu Asn Tyr Gly Ala Leu Phe Asp
915 920 925

Leu Phe Glu Gln Lys Leu Arg Lys Leu Thr Gln His Ile Asp Arg Ser
930 935 940

Asn Leu Lys Pro Glu Glu Ala Ile Arg Phe Arg Gln Asp Ile Ala His
945 950 955 960

Leu Arg Asp Ile Ser Asn His Leu Ala Ser Asn Ser Ala His Ala Asn
965 970 975

Glu Gly Ala Gly Glu Leu Leu Arg Gln Pro Ser Leu Glu Ser Val Ala
980 985 990

Ser His Arg Ser Ser Met Ser Ser Ser Ser Lys Ser Ser Lys Gln Glu
995 1000 1005

Lys Ile Ser Leu Ser Ser Phe Gly Lys Asn Lys Lys Ser Trp Ile Arg
1010 1015 1020

Ser Ser Leu Ser Lys Phe Thr Lys Lys Lys Asn Lys Asn Tyr Asp Glu
1025 1030 1035 1040

Ala His Met Pro Ser Ile Ser Gly Ser Gln Gly Thr Leu Asp Asn Ile
1045 1050 1055

Asp Val Ile Glu Leu Lys Gln Glu Leu Lys Glu Arg Asp Ser Ala Leu
1060 1065 1070

Tyr Glu Val Arg Leu Asp Asn Leu Asp Arg Ala Arg Glu Val Asp Val
1075 1080 1085

Leu Arg Glu Thr Val Asn Lys Leu Lys Thr Glu Asn Lys Gln Leu Lys
1090 1095 1100

Lys Glu Val Asp Lys Leu Thr Asn Gly Pro Ala Thr Arg Ala Ser Ser
1105 1110 1115 1120

Arg Ala Ser Ile Pro Val Ile Tyr Asp Asp Glu His Val Tyr Asp Ala
1125 1130 1135

Ala Cys Ser Ser Thr Ser Ala Ser Gln Ser Ser Lys Arg Ser Ser Gly
1140 1145 1150

Cys Asn Ser Ile Lys Val Thr Val Asn Val Asp Ile Ala Gly Glu Ile
1155 1160 1165

Ser Ser Ile Val Asn Pro Asp Lys Glu Ile Ile Val Gly Tyr Leu Ala
1170 1175 1180

Met Ser Thr Ser Gln Ser Cys Trp Lys Asp Ile Asp Val Ser Ile Leu
1185 1190 1195 1200

Gly Leu Phe Glu Val Tyr Leu Ser Arg Ile Asp Val Glu His Gln Leu
1205 1210 1215

Gly Ile Asp Ala Arg Asp Ser Ile Leu Gly Tyr Gln Ile Gly Glu Leu
1220 1225 1230

Arg Arg Val Ile Gly Asp Ser Thr Thr Met Ile Thr Ser His Pro Thr
1235 1240 1245

Asp Ile Leu Thr Ser Ser Thr Thr Ile Arg Met Phe Met His Gly Ala
1250 1255 1260

Ala Gln Ser Arg Val Asp Ser Leu Val Leu Asp Met Leu Leu Pro Lys
1265 1270 1275 1280

Gln Met Ile Leu Gln Leu Val Lys Ser Ile Leu Thr Glu Arg Arg Leu
1285 1290 1295

Val Leu Ala Gly Ala Thr Gly Ile Gly Lys Ser Lys Leu Ala Lys Thr
1300 1305 1310

Leu Ala Ala Tyr Val Ser Ile Arg Thr Asn Gln Ser Glu Asp Ser Ile
1315 1320 1325

Val Asn Ile Ser Ile Pro Glu Asn Asn Lys Glu Glu Leu Leu Gln Val
1330 1335 1340

Glu Arg Arg Leu Glu Lys Ile Leu Arg Ser Lys Glu Ser Cys Ile Val
1345 1350 1355 1360

Ile Leu Asp Asn Ile Pro Lys Asn Arg Ile Ala Phe Val Val Ser Val
1365 1370 1375

Phe Ala Asn Val Pro Leu Gln Asn Asn Glu Gly Pro Phe Val Val Cys
1380 1385 1390

Thr Val Asn Arg Tyr Gln Ile Pro Glu Leu Gln Ile His His Asn Phe
1395 1400 1405

Lys Met Ser Val Met Ser Asn Arg Leu Glu Gly Phe Ile Leu Arg Tyr
1410 1415 1420

Leu Arg Arg Arg Ala Val Glu Asp Glu Tyr Arg Leu Thr Val Gln Met
1425 1430 1435 1440

Pro Ser Glu Leu Phe Lys Ile Ile Asp Phe Phe Pro Ile Ala Leu Gln
1445 1450 1455

Ala Val Asn Asn Phe Ile Glu Lys Thr Asn Ser Val Asp Val Thr Val
1460 1465 1470

```
Gly Pro Arg Ala Cys Leu Asn Cys Pro Leu Thr Val Asp Gly Ser Arg
        1475                1480                1485

Glu Trp Phe Ile Arg Leu Trp Asn Glu Asn Phe Ile Pro Tyr Leu Glu
    1490                1495                1500

Arg Val Ala Arg Asp Gly Lys Lys Thr Phe Gly Arg Cys Thr Ser Phe
1505                1510                1515                1520

Glu Asp Pro Thr Asp Ile Val Ser Lys Lys Trp Pro Trp Phe Asp Gly
                1525                1530                1535

Glu Asn Pro Glu Asn Val Leu Lys Arg Leu Gln Leu Gln Asp Leu Val
            1540                1545                1550

Pro Ser Pro Ala Asn Ser Ser Arg Gln His Phe Asn Pro Leu Glu Ser
        1555                1560                1565

Leu Ile Gln Leu His Ala Thr Lys His Gln Thr Ile Asp Asn Ile
    1570                1575                1580
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ATAAGAATGC GGCCGCCGCC ATGACGACGT CAAATGTAGA ATTGATA 47

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGAATTCCAA CCATATGACG ACGTCAAATG TAGAATTGAT A 41

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CGCGGATCCT CAAACCGCGG GTGGCATAAT GGATG 35

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Lys Lys Asp Pro Pro Pro Ala Val Pro Pro Arg Asp Thr
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Thr Thr Asp Val Pro Pro Leu Pro Pro Leu Lys Ser
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Glu Val Pro Val Pro Pro Pro Val Pro Pro Arg Arg
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
His Leu Asp Ser Pro Pro Ala Ile Pro Pro Arg
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
His Ser Ile Ala Gly Pro Pro Val Pro Pro Arg
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
Tyr Arg Ala Val Pro Pro Pro Leu Pro Pro Arg Arg Lys
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
Gly Glu Leu Ser Pro Pro Pro Ile Pro Pro Arg Leu Asn
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```
Ala Pro Ala Val Pro Pro Ala Arg Pro Gly Ser
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
Pro Ala Val Pro Pro Ala Arg Pro
1               5
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Pro Pro Arg Pro Leu Pro Val Ala Pro Gly Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Pro Ala Pro Ala Pro Pro Lys Pro Pro Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Pro Pro Asp Asn Gly Pro Pro Pro Leu Pro Thr Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Pro Pro Gln Met Pro Leu Pro Glu Ile Pro Gln Gln Trp
1 5 10

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```
Ala Pro Thr Met Pro Pro Pro Leu Pro Pro Val Pro Pro
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

```
Phe Pro Ala Tyr Pro Pro Pro Pro Val Pro Val Pro
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
Leu Leu Phe Leu Leu Ser Thr Tyr Lys Gln Lys Leu Arg Gln Leu Lys
1               5                   10                  15

Lys Asp Gln Lys Lys Leu Glu Gln Leu Pro Thr Ser
            20                  25
```

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
Glu Thr Val Asn Val Asn Lys Leu Lys Thr Glu Asn Lys Gln Leu Lys
1               5                   10                  15

Lys Glu Val Asp Lys Leu Thr Asn Gly Pro Ala Thr
            20                  25
```

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10443 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "plasmid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GGCCGCCGCC ATGACGACGT CAAATGTAGA ATTGATACCA ATCTACACGG ATTGGGCCAA 60

TCGGCACCTT TCGAAGGGCA GCTTATCAAA GTCGATTAGG GATATTTCCA ATGATTTTCG 120

CGACTATCGA CTGGTTTCTC AGCTTATTAA TGTGATCGTT CCGATCAACG AATTCTCGCC 180

TGCATTCACG AAACGTTTGG CAAAAATCAC ATCGAACCTG GATGGCCTCG AAACGTGTCT 240

CGACTACCTG AAAAATCTGG GTCTCGACTG CTCGAAACTC ACCAAAACCG ATATCGACAG 300

CGGAAACTTG GGTGCAGTTC TCCAGCTGCT CTTCCTGCTC TCCACCTACA AGCAGAAGCT 360

TCGGCAACTG AAAAAAGATC AGAAGAAATT GGAGCAACTA CCCACATCCA TTATGCCACC 420

CGCGGTTTCT AAATTACCCT CGCCACGTGT CGCCACGTCA GCAACCGCTT CAGCAACTAA 480

CCCAAATTCC AACTTTCCAC AAATGTCAAC ATCCAGGCTT CAGACTCCAC AGTCAAGAAT 540

ATCGAAAATT GATTCATCAA AGATTGGTAT CAAGCCAAAG ACGTCTGGAC TTAAACCACC 600

CTCATCATCA ACCACTTCAT CAAATAATAC AAATTCATTC CGTCCGTCGA GCCGTTCGAG 660

TGGCAATAAT AATGTTGGCT CGACGATATC CACATCTGCG AAGAGCTTAG AATCATCATC 720

AACGTACAGC TCTATTTCGA ATCTAAACCG ACCTACCTCC CAACTCCAAA AACCTTCTAG 780

ACCACAAACC CAGCTAGTTC GTGTTGCTAC AACTACAAAA ATCGGAAGCT CAAAGCTAGC 840

CGCTCCGAAA GCCGTGAGCA CCCCAAAACT TGCTTCTGTG AAGACTATTG GAGCAAAACA 900

AGAGCCCGAT AACAGCGGTG GTGGTGGTGG TGGAATGCTG AAATTAAAGT TATTCAGTAG 960

CAAAAACCCA TCTTCCTCAT CGAATAGCCC ACAACCTACG AGAAAGGCGG CGGCGGTGCC 1020

TCAACAACAA ACTTTGTCGA AAATCGCTGC CCCAGTGAAA AGTGGCCTGA AGCCGCCGAC 1080

CAGTAAGCTG GGAAGTGCCA CGTCTATGTC GAAGCTTTGT ACGCCAAAAG TTTCCTACCG 1140

TAAAACGGAC GCCCCAATCA TATCTCAACA AGACTCGAAA CGATGCTCAA AGAGCAGTGA 1200

AGAAGAGTCC GGATACGCTG GATTCAACAG CACGTCGCCA ACGTCATCAT CGACGGAAGG 1260

TTCCCTAAGC ATGCATTCCA CATCTTCCAA GAGTTCAACG TCAGACGAAA AGTCTCCGTC 1320

ATCAGACGAT CTTACTCTTA ACGCCTCCAT CGTGACAGCT ATCAGACAGC CGATAGCCGC 1380

AACACCGGTT TCTCCAAATA TTATCAACAA GCCTGTTGAG GAAAAACCAA CACTGGCAGT 1440

GAAAGGAGTG AAAAGCACAG CGAAAAAAGA TCCACCTCCA GCTGTTCCGC CACGTGACAC 1500
CCAGCCAACA ATCGGAGTTG TTAGTCCAAT TATGGCACAT AAGAAGTTGA CAAATGACCC 1560
CGTGATATCT GAAAAACCAG AACCTGAAAA GCTCCAATCA ATGAGCATCG ACACGACGGA 1620
CGTTCCACCG CTTCCACCTC TAAAATCAGT TGTTCCACTT AAAATGACTT CAATCCGACA 1680
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(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7474 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "plasmid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

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(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13414 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "plasmid"

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:11582
(D) OTHER INFORMATION:/note= "N is A,G,C or T"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

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GAAACGTTTG GCAAAAATCA CATCGAACCT GGATGGCCTC GAAACGTGTC TCGACTACCT 240
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GACATAATGG GCTAAACAAG ACTACACCAA TTACACTGCC TCATTGATGG TGGTACATAA 12420
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GAATAAGTGC GACATCATCA TCGGAAGAGA GTAGTAACAA AGGTCAAAGA CAGTTGACTG 13380

TATCGCCGGA ATTGCAATAC CCAGCTTTGA CTCA 13414

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "plasmid"

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:8456
(D) OTHER INFORMATION:/note= "N is A,C,G, or T"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

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CGGTGCCGCA CAGAGTCGCG TAGACAGTCT GGTCCTTGAT ATGCTTCTTC CAAAGCAAAT 720
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AAAAAAAAAA AAAAAAAAAA AAAAGGCCTA TGCGGCCGGG CCATGGAGGC CGAATTCCCG 1920
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SUBSTITUTE SHEET (RULE 26)

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CGGAATTGCA ATACCCAGCT TTGACTCA 10288

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7625 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "plasmid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

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GTTGCTTTCT	CAGGTATAGC	ATGAGGTCGC	TCTTATTGAC	CACACCTCTA	CCGGCATGCC	2400
CGAAATTCCC	CTACCCTATG	AACATATTCC	ATTTTGTAAT	TTCGTGTCGT	TTCTATTATG	2460
AATTTCATTT	ATAAAGTTTA	TGTACAAATA	TCATAAAAAA	AGAGAATCTT	TTTAAGCAAG	2520
GATTTTCTTA	ACTTCTTCGG	CGACAGCATC	ACCGACTTCG	GTGGTACTGT	TGGAACCACC	2580
TAAATCACCA	GTTCTGATAC	CTGCATCCAA	AACCTTTTTA	ACTGCATCTT	CAATGGCCTT	2640
ACCTTCTTCA	GGCAAGTTCA	ATGACAATTT	CAACATCATT	GCAGCAGACA	AGATAGTGGC	2700
GATAGGGTCA	ACCTTATTCT	TTGGCAAATC	TGGAGCAGAA	CCGTGGCATG	GTTCGTACAA	2760
ACCAAATGCG	GTGTTCTTGT	CTGGCAAAGA	GGCCAAGGAC	GCAGATGGCA	ACAAACCCAA	2820
GGAACCTGGG	ATAACGGAGG	CTTCATCGGA	GATGATATCA	CCAAACATGT	TGCTGGTGAT	2880
TATAATACCA	TTTAGGTGGG	TTGGGTTCTT	AACTAGGATC	ATGGCGGCAG	AATCAATCAA	2940
TTGATGTTGA	ACCTTCAATG	TAGGAAATTC	GTTCTTGATG	GTTTCCTCCA	CAGTTTTTCT	3000
CCATAATCTT	GAAGAGGCCA	AAACATTAGC	TTTATCCAAG	GACCAAATAG	GCAATGGTGG	3060
CTCATGTTGT	AGGGCCATGA	AAGCGGCCAT	TCTTGTGATT	CTTTGCACTT	CTGGAACGGT	3120

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AATACCTCCC ACTAATTCTC TGACAACAAC GAAGTCAGTA CCTTTAGCAA ATTGTGGCTT 3240
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GTTTGAAAAA TTAGCGCTCT CGCGTTGCAT TTTTGTTCTA CAAAATGAAG CACAGATGCT 7620

TCGTT 7625

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9642 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "plasmid"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

ATGACCATGA TTACGCCAAG CTTGTCTTCT TCTAAATTCC CATAAAATCC CGAAACTCCT 60

TCCCTCTATC TTCTTTTTCT TCTCGTTTTC AAATGTTTCT CTCTATCCCA TTCTCTCATC 120

AATTGAGTGG GATGAGGCTA TCTCTGCCTC TCTTCTGAAT CTCTGAACCA TCTTACATTA 180

CACTGTGGAT GACGAGCCCC ACAGGCTCCC TTGCATCAGA TACTGCCATT GGGGATGGCA 240

AAGAAGAGAG AAGGTATTGT GAGGATATAT TTTTCTAAGA AAAAACGTTT GAAGAAAAGA 300

AGATGAAGAA GATCTGCTTG ATTCATTGCA CAAGTTAGAA GTAACAGGGG TCTATATTTC 360

GAAGAACTTA AAGGGAATGC AACTGAACAT AAAATTAAAC AAAGGGATTG AATCCTGCAG 420

TGAGTATTTT CGGTTTTTCA CTGGTTCTCT GTAAAAAGAG TAATGCAAAG GGCAAGTTAA 480

CTTAGGTCGT AAATGTATTG AATTTGCTTA AAATCTGAAG ATCTAGTGGT GAACCGTGGA 540

AGATTATCAA GAGGAGGCTG AAGATCTGTT TAAGAACCAT TAATCAAACT GGTATTCTAT 600

TTTCACTGGT TGTATGTAAA CATTCTATCT TATTCCTTTT ATCACTGTTC TGCACTTTCC 660

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GCTTGGAGCG AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCAT TGAGAAAGCG 9060

CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG 9120

GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT 9180

TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT 9240

GGAAAAACGC CAGCAACGCG GCCTTTTTAC GGTTCCTGGC CTTTTGCTGG CCTTTTGCTC 9300

ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT 9360

GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG 9420

CGGAAGAGCG CCCAATACGC AAACCGCCTC TCCCCGCGCG TTGGCCGATT CATTAATGCA 9480

GCTGGCACGA CAGGTTTCCC GACTGGAAAG CGGGCAGTGA GCGCAACGCA ATTAATGTGA 9540

GTTAGCTCAC TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT 9600

GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CT 9642

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp Ala
1 5 10 15

Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg Asp Ile
20 25 30

Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln Leu Ile Asn Val
35 40 45

Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr Lys Arg Leu Ala
50 55 60

Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr Cys Leu Asp Tyr Leu
65 70 75 80

Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu Thr Lys Thr Asp Ile Asp
85 90 95

Ser Gly Asn Leu Gly Ala Val Leu Gln Leu Leu Phe Leu Leu
100 105 110

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Lys Gln Lys Leu Arg Gln Leu Lys Lys Asp Gln Lys Lys Leu Glu Gln
1 5 10 15

Leu Pro Thr Ser
20

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Asp Pro Pro Pro Ala Val Pro Pro Arg
1 5

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

```
Asp Val Pro Pro Leu Pro Pro Leu Lys
1               5
```

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

```
Lys Lys Lys Asn Lys
1               5
```

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

```
Lys Thr Glu Asn Lys Gln Leu Lys Lys Glu Val Asp Lys Leu Thr Asn
1               5                   10                  15

Gly Pro Ala Thr
            20
```

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

```
Gly Ala Thr Gly Ile Gly Lys Ser
1               5
```

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 58 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

```
Met Ser Glu Glu Pro Thr Pro Val Ser Gly Asn Asp Lys Gln Leu Leu
1               5                   10                  15

Asn Lys Ala Trp Glu Ile Thr Gln Lys Lys Thr Phe Thr Ala Trp Cys
            20                  25                  30

Asn Ser His Leu Arg Lys Leu Gly Ser Ser Ile Glu Gln Ile Asp Thr
        35                  40                  45

Asp Phe Thr Asp Gly Ile Lys Leu Ala Gln
    50                  55
```

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

```
Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp Ala
1               5                   10                  15

Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg Asp Ile
            20                  25                  30

Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln
        35                  40
```

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

```
Phe Glu Arg Ser Arg Ile Lys Ala Leu Ala Asp Glu Arg Glu Val Val
1               5                   10                  15

Gln Lys Lys Thr Phe Thr Lys Trp Val Asn Ser His Leu Ala Arg Val
            20                  25                  30

Ser Cys Arg Ile Thr Asp Leu Tyr Lys Asp Leu Arg Asp Gly Arg Met
        35                  40                  45

Leu Ile Lys
    50
```

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

```
Leu Leu Glu Val Ile Ser Asn Asp Pro Val Phe Lys Val Asn Lys Thr
1               5                   10                  15

Pro Lys Leu Arg Arg Ile His Asn Ile Gln Asn Val Gly Leu Cys Leu
            20                  25                  30

Lys His Ile Glu Ser His Gly Val Lys Leu Val Gly Ile Gly Ala Glu
        35                  40                  45

Glu Leu Val Asp Lys Asn Leu Lys Met Thr Leu
    50                  55
```

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

```
Leu Ile Asn Val Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr
1               5                   10                  15

Lys Arg Leu Ala Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr Cys
            20                  25                  30

Leu Asp Tyr Leu Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu Thr Lys
        35                  40                  45

Thr Asp Ile Asp Ser Gly Asn Leu Gly Ala Val Leu
    50                  55                  60
```

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

Leu Leu Glu Val Leu Ser Gly Glu Met Leu Pro Lys Pro Thr Lys Gly
1 5 10 15

Lys Met Arg Ile His Cys Leu Glu Asn Val Asp Lys Ala Leu Gln Phe
20 25 30

Leu Lys Glu Gln Arg Val His Leu Glu Asn Met Gly Ser His Asp Ile
35 40 45

Val Asp Gly Asn His Arg Leu Val Leu
50 55

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Gly Met Ile Trp Thr Ile Ile Leu Arg Phe Ala Ile Gln Asp Ile Ser
1 5 10 15

Ile Glu Glu Leu Ser Ala Lys Glu Ala Leu Leu Leu Trp Cys Gln Arg
20 25 30

Lys Thr Glu Gly Tyr Asp Arg Val Lys Val
35 40

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

```
Gln Leu Leu Phe Leu Leu Ser Thr Tyr Lys Gln Lys Leu Arg Gln Leu
1               5                   10                  15

Lys Lys Asp Gln Lys Lys Leu Glu Gln Leu Pro Thr Ser Ile Met Pro
            20                  25                  30

Pro Ala Val Ser Lys Leu Pro Ser Pro Arg Val Ala Thr Ser
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

```
Gly Leu Ile Trp Thr Ile Ile Leu Arg Phe Gln Ile Gln Asp Ile Val
1               5                   10                  15

Val Gln Thr Gln Glu Gly Arg Glu Thr Arg Ser Ala Lys Asp Ala Leu
            20                  25                  30

Leu Gln Phe Leu Lys Glu Gln Arg Val His Leu Glu Asn Met Gly Ser
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 100 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cosmid DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

```
GATCAGAAGA AATTGGAGCA ACTACCCACA TCCATTATGC CACCCGCGGT TTCTAAGTGA    60

GTTTAATTTT GAGTTTACGA CTACAAAAAT GTGTTCTTTA                          100
```

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 91 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cosmid DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

CCGCCTTCTG ACTTCGTGAC GACAGTCTCG ACACGTGGGG TTGCAGGTAG GAGTGGATGA 60

GTCGAAACTG ATAAGATAGT CATTTGAGAT C 91

CLAIMS:

1. A cDNA encoding an UNC-53 protein of C. elegans or a functional equivalent derivative fragment or bioprecursor of said protein, which cDNA comprises at least from nucleotide position 431 to nucleotide position 4647 of the sequence shown in Figure 1.

2. A cDNA as claimed in claim 1 comprising at least from nucleotide position 431 to the 3′ end of the sequence shown in Figure 1.

3. A cDNA as claimed in Claim 1 comprising at least from nucleotide position 64 to nucleotide position 4647 of the sequence as shown in Figure 1.

4. A cDNA as claimed in claim 3 comprising at least from nucleotide position 64 to the 3′ end of the sequence shown in Figure 1.

5. A cDNA as claimed in Claims 1 to 4 comprising the nucleotide sequence shown in Figure 1.

6. A cDNA encoding an UNC-53 protein of C. elegans or a functional equivalent, derivative, fragment or bioprecursor of said protein, which cDNA comprises at least from nucleotide position 431 to nucleotide position 4812 of the 7A variant of the sequence shown in Figure 2.

7. A cDNA as claimed in claim 6 comprising at least

from nucleotide position 431 to the 3′ end of the 7A variant of the sequences shown in figure 2.

8. A cDNA as claimed in Claim 6 comprising at least from nucleotide position 64 to nucleotide position 4812 of the sequence shown in Figure 2.

9. A cDNA as claimed in claim 8 comprising at least from nucleotide position 64 to the 3′ end of the 7A variant of the sequence shown in figure 2.

10. A cDNA as claimed in any of claims 6 to 9 comprising the nucleotide sequence of the 7A variant of the sequence shown in Figure 2.

11. A DNA expression vector which comprises a cDNA as claimed in any one of Claims 1 to 10.

12. A host cell transformed or transfected with the vector of Claim 11.

13. A host cell as claimed in Claim 12 which is a bacterial, an animal, a plant or an insect cell.

14. A transgenic cell comprising a transgene capable of expressing UNC-53 protein of C. elegans or a functional equivalent, derivative, fragment or bioprecursor of said protein.

15. A transgenic cell as claimed in Claim 14 which

cell is a <u>C. elegans</u> cell, an N4 neuroblastoma cell or an MCF-7 breast carcinoma cell.

16. A transgenic organism comprising a transgene capable of expressing UNC-53 protein of <u>C. elegans</u> or a functional equivalent, derivative, fragment or bioprecursor of said protein.

17. A transgenic organism as claimed in Claim 16 wherein said organism is <u>C. elegans</u>.

18. A transgenic organism as claimed in Claim 16 wherein said organism is an insect, a non-human mammal or a plant.

19. A mutant of <u>C. elegans</u> which comprises an induced mutation in the wild-type unc-53 gene, which mutation affects the regulation of cell motility or the shape or direction of cell migration.

20. An UNC-53 protein encoded by the cDNA of Claim 1 and which protein has the amino acid sequence shown in Figure 4 from amino acid position 135 to amino acid position 1528.

21. An UNC-53 protein encoded by the cDNA sequence of any of Claims 2 to 5 and which protein has the amino acid sequence shown in Figure 4.

22. An UNC-53 protein encoded by the cDNA sequence of Claim 6 and which protein has the amino acid

sequence shown in Figure 6 from amino acid position 135 to amino acid position 1583.

23. An UNC-53 protein encoded by the cDNA sequence according to any of Claims 7 to 10 and which protein has the amino acid sequence shown in Figure 6.

24. An UNC-53 protein of C. elegans, or a functional equivalent, derivative, fragment or bioprecursor of said protein, for use as a medicament to promote neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

25. An UNC-53 protein as claimed in any one of Claims 20 to 23 for use as a medicament to promote neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

26. Use of an UNC-53 protein of C. elegans, or a functional equivalent, derivative, fragment or bioprecursor of said protein in the manufacture of a medicament for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

27. Use of an UNC-53 protein as claimed in any one of Claims 20 to 23 in the manufacture of a medicament for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative or acute traumatic injuries.

28. A pharmaceutical composition comprising an UNC-53 protein of C. elegans, a functional equivalent, derivative, bioprecursor or fragment of said protein and an acceptable carrier, diluent or excipient therefor.

29. A pharmaceutical composition as claimed in Claim 28 which comprises an UNC-53 protein as claimed in any one of Claims 20 to 23.

30. A nucleic acid sequence encoding an UNC-53 protein of C. elegans or a functional fragment, equivalent, derivative or bioprecursor of said protein, for use as a medicament to promote neuronal regeneration, vascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

31. A nucleic acid sequence for use as claimed in Claim 27 wherein said sequence is a cDNA sequence as claimed in any one of Claims 1 to 10 or a functional fragment of said nucleic acid sequence.

32. Use of a nucleic acid sequence encoding and UNC-53 protein of C. elegans or a functional equivalent fragment, derivative or bioprecursor of said protein, in the manufacture of a medicament to promote neuronal regeneration, vascularization or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

33. Use of a nucleic acid sequence as claimed in Claim 32 wherein said sequence is a cDNA sequence as

claimed in any one of Claims 1 to 10 or a functional fragment of said nucleic acid sequence.

34. A pharmaceutical composition comprising a nucleic sequence acid encoding an UNC-53 protein of C. elegans or a functional equivalent, derivative fragment or bioprecursor of said protein and an acceptable carrier, diluent, or excipient therefor.

35. A pharmaceutical composition as claimed in Claim 34 wherein said nucleic acid sequence is a cDNA sequence as claimed in any one of Claims 1 to 10.

36. A method of determining whether a compound is an inhibitor or an enhancer of the regulation of cell shape or motility or the direction of cell migration, which method comprises contacting said compound with a transgenic cell as claimed in Claims 14 or 15 and screening for a phenotypic change in said cell.

37. A method as claimed in Claim 36 wherein said compound is an inhibitor or an enhancer of a protein of the signal transduction pathway of said transgenic cell of which pathway UNC-53 protein or a functional equivalent, fragment or bioprecursor thereof is a component or said compound is an inhibitor or an enhancer of a parallel or redundant signal transduction pathway in said cell.

38. A method as claimed in Claim 36 or 37 wherein said protein is UNC-53 protein or a functional equivalent, fragment, derivative or bioprecursor thereof.

39. A method as claimed in any of Claims 36 to 38 wherein said phenotypic change to be screened is a change in cell shape or a change in cell motility.

40. A method as claimed in any of claims 36 to 38 wherein said phenotypic change to be screened is a change in filipodia outgrowth, ruffling behaviour, cell adhesion or the length of neurite growth.

41. A method as claimed in any of Claims 36 to 40 wherein said transgenic cell is an N4 neuroblastoma cell and the phenotypic change to be screened is the length of neurite growth.

42. A method as claimed in any of Claims 36 to 40 wherein said transgenic cell is an MCF-7 breast carcinoma cell and the phenotypic change to be screened is the extent of phagokinesis.

43. A method of determining whether a compound is an inhibitor or an enhancer of the regulation of cell shape or motility or of the direction of cell migration which method comprises administering said compound to a transgenic organism as claimed in any one of Claims 16 to 20, or a mutant organism as claimed in Claim 19, and screening for a phenotypic change in said organism.

44. A method as claimed in Claim 43 wherein said compound is an inhibitor or enhancer of a protein of the signal transduction pathway of said transgenic or mutant organisms, of which pathway UNC-53 protein or a functional equivalent, derivative or bioprecursor

thereof is a component or said compound is an inhibitor or an enhancer of a parallel or redundant signal transduction pathway in said cell.

45. A method as claimed in Claim 44 wherein said protein of the signal transduction pathway is UNC-53 protein itself or a functional equivalent, fragment, derivative or bioprecursor of said protein.

46. A compound which is identifiable by the method according to any one of Claims 36 to 45 as an enhancer of the regulation of cell shape or motility or the direction of cell migration for use as a medicament for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

47. Use of a compound identifiable by the method of any one of Claims 36 to 45 as an enhancer of the regulation of cell shape or motility or the direction of cell migration in C. elegans in the manufacture of a medicament for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

48. A pharmaceutical composition comprising the compound as claimed in Claim 46 and an acceptable carrier, diluent or excipient therefor.

49. A compound which is identifiable by the method according to any one of Claims 36 to 45 as an inhibitor of the regulation of cell motility or shape

or the direction of cell migration of C. elegans for use as a medicament for alleviating the spread of disease inducing cells or metastasis.

50. Use of a compound identifiable by the method according to any one of Claims 36 to 45 in the manufacture of a medicament for alleviating the spread of disease inducing cells or metastasis.

51. A pharmaceutical composition comprising the compound as claimed in Claim 49 and an acceptable carrier diluent or excipient therefor.

52. A transgenic cell which has been constructed to comprise a promoter sequence of an unc-53 gene of C. elegans fused to a nucleic acid sequence encoding a reporter molecule.

53. A transgenic cell as claimed in Claim 52 wherein said reporter molecule is green fluorescent protein (GFP).

54. A method of determining whether a compound is an inhibitor or an enhancer of transcription of an unc-53 gene in C. elegans or a functional fragment of said gene, which method comprises the steps of (a) contacting said compound with a transgenic cell according to Claim 52 and (b) monitoring of said reporter molecule and comparing the results obtained from said monitoring step with a control comprising a transgenic cell as claimed in Claim 48, which cell has not been contacted with said compound.

55. A method as claimed in Claim 54 wherein said reporter molecule detected is mRNA.

56. A method as claimed in Claim 54 wherein said reporter molecule detected is green fluorescent protein (GFP).

57. A compound which is identifiable by the method according to any one of Claims 54 to 56, as an enhancer of transcription of an unc-53 gene of C. elegans or a functional fragment of said gene for use in promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

58. Use of a compound which is identifiable by the method of any one of Claims 54 to 56 as an enhancer of transcription of an unc-53 gene of C. elegans or a functional fragment of said gene in the manufacture of a medicament for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

59. A pharmaceutical composition which comprises the compound of Claim 57 and an acceptable carrier, diluent or excipient therefor.

60. A compound which is identifiable by the method of any one of Claims 54 to 56 as an inhibitor of transcription of an unc-53 gene of C. elegans or a functional fragment of said gene for use in

alleviating the spread of disease inducing cells or metastasis.

61. Use of a compound which is identifiable by the method of any one of Claims 54 to 56 as an inhibitor of transcription of an unc-53 gene of C. elegans or a functional fragment of said gene in the manufacture of a medicament for alleviating spread of disease inducing cells or metastasis.

62. A pharmaceutical composition which comprises the compound of Claim 60 and an acceptable carrier, diluent or excipient therefor.

63. A kit for determining whether a compound is an enhancer or an inhibitor of the regulation of cell motility or shape or the direction of cell migration which kit comprises at least a plurality of transgenic cells as claimed in any one of Claims 14 or 15 and a plurality of wild-type cells of the same cell or cell-line.

64. A kit for determining whether a compound is an inhibitor or an enhancer of transcription of an unc-53 gene of C. elegans or a functional fragment of said gene which kit comprises at least a plurality of transgenic cells as claimed in Claims 52 or 53 and means for monitoring the reporter molecule.

65. A kit for determining whether a compound is an enhancer or an inhibitor of the activity of UNC-53 protein or a functional equivalent, derivative, fragment or bioprecusor of said protein, which kit

comprises at least, one mutant organism of C. elegans as claimed in claim 10 or a transgenic organism as claimed in any of claims 16 to 18 and a wild type organism of C. elegans.

66. An oligonucleotide probe which comprises the carboxy-terminal 1.5 kb of the coding nucleic acid sequence shown in Figure 1 or a fragment thereof comprising between 18 and 24 base pairs.

67. An oligonucleotide probe comprising a nucleic acid sequence encoding the amino acid sequence as numbered 1 to 110, 114 to 133, 487 to 495, 537 to 545, 1032 to 1037, 1097 to 1116 or 1300 to 1307 shown in Figure 3 or a fragment thereof.

68. A probe as claimed in Claim 66 or 67 which is labelled for detection.

69. A method of identifying homologues of a C. elegans unc-53 gene or a functional fragment thereof which method comprises hybridizing to a C. elegans DNA library an oligonucleotide probe as claimed in any one of Claims 66 to 68 under appropriate conditions of stringency to identify genes having statistically significant homology with the cDNA of any one of Claims 1 to 10.

70. A method of identifying a protein which is active in the signal transduction pathway of a cell of which an UNC-53 protein or a functional equivalent, fragment or bioprecursor of said UNC-53 protein is a component, which method comprises:

component, which method comprises:

(a) contacting an extract of said cell with an antibody to the UNC-53 protein of C.elegans or a functional equivalent, fragment, derivative or bioprecursor of said protein,

(b) identifying the antibody/UNC-53 complex, and

(c) analysing the complex to identify any protein bound to the UNC-53 protein other than the antibody.

71. A method of identifying a further protein which is active in the signal transduction pathway of a cell of which an UNC-53 protein or a functional equivalent, fragment or bioprecursor of said UNC-53 protein is a component which method comprises:

(a) forming an antibody to the identified protein bound to the UNC-53 protein in Claim 65,

(b) contacting a cell extract with said antibody and identifying the antibody/protein complex,

(c) analysing the complex to identify any further protein bound to the first protein other than the antibody, and

(d) optionally repeating steps (a) to (c) to identify further proteins in said pathway.

72. A method of identifying a protein which is active in the signal transduction pathway of a cell of which an UNC-53 protein or a functional equivalent, fragment or bioprecursor of said UNC-53 protein is a component, which method comprises

(a) contacting an extract of said cell with UNC-53 protein of C. elegans or a functional equivalent, derivative or bioprecursor of said UNC-53 protein

(b) identifying UNC-53 protein/protein complex formed and

(c) analysing the complex to identify any protein bound to the UNC-53 protein other than another UNC-53 protein.

73. A method according to claim 72 which further comprises contacting a cell extract with any protein identified from step (c) not being UNC-53 protein and repeating steps (b) and (c) so as to identify any further protein involved in the signal transduction pathway of said cell.

74. A method of identifying a protein involved in the signal transduction pathway of C. elegans which method comprises:

(a) constructing at least two nucleotide vectors, the first of which comprises a nucleotide segment encoding for a DNA binding domain of GAL4 protein fused to a sequence encoding UNC-53 protein of C. elegans or a functional equivalent, derivative, fragment or bioprecursor thereof, the second vector comprising a nucleotide sequence encoding a protein binding domain of GAL4 protein fused to a nucleotide sequence encoding a protein to be tested,

(b) co-transforming each of said vectors into a yeast cell being deficient for transcription of genes encoding galactose metabolites, wherein

interaction between said test protein and said UNC-53 protein leads to transcription of said galactose metabolite genes.

75. A protein identified by the method, of any one of claims 70 to 74 for use as a medicament to promote neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degeroactive diseases or acute traumatic injuries.

76. Use of a protein identified by the methods of any one of claims 70 to 74 in the manufacture of a medicament for promoting neuronal regeneration, revasculerisation or wound healing, or for treatment of chronic neurodegenerative diseases or acute traumatic injuries.

77. A pharmaceutical composition comprising a protein identified by the methods of any one of Claims 70 to 74 and an acceptable carrier diluent, or excipient therefor.

78. A process for producing an UNC-53 protein of C. elegans or a functional equivalent fragment, derivative or bioprecursor of said UNC-53 protein which process comprises culturing the transfected or transformed cells of Claim 12 or Claim 13 and recovering the expressed UNC-53 protein.

79. A process for producing an UNC-53 protein of C. elegans or a functional equivalent fragment, derivative or bioprecursor of said protein which process comprises culturing an insect cell transfected

with a recombinant Baculovirus vector, said vector comprising a DNA insert encoding said UNC-53 protein or a functional equivalent, fragment or bioprecursor thereof, downstream of the Baculovirus polyhedrin promoter, and recovering the expressed UNC-53 protein.

80. A hybridoma cell line deposited under the LMBP Accession No. 1383CB.

81. Monoclonal antibody 16-48-2 obtainable from the hybridoma deposited under the LMBP Accession No. 1383CB.

82. Plasmid pTB54 deposited under the LMBP Accession No. 3296.

83. Plasmid pBT112 deposited under the Accession No. 3295.

84. Plasmid pTB72 deposited under the LMBP Accession No. 3486.

85. Transgenic cell-line of C.elegans designated TB4EX25 and deposited under the LMBP Accession No. 1384CB.

86. Transgenic cell-line of C. elegans designated TBAIn76 and deposited under the Accession No. 1385CB.

87. A transgenic cell-line of MCF-7 breast carcinoma

cells deposited under the LMBP Accession No. 1550CB.

88. A transgenic cell-line of N4 neuroblastoma cells deposited under LMBP Accession No. 1549CB.